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*12,361 volunteer abstracts, 16 symposia abstracts, 20 history of neuroscience abstracts, and 41 teaching of neuroscience abstracts.
1995 Program Committee

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Michael J. Zigmond, Ph.D., ex officio
University of Pittsburgh
SOCIETY FOR NEUROSCIENCE POLICIES ON THE USE OF ANIMALS AND HUMANS IN NEUROSCIENCE RESEARCH

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

POLICY ON THE USE OF ANIMALS IN NEUROSCIENCE RESEARCH

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

Introduction

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

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

General Policy

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

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

Local Committee Review

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

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

Recommended References


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


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


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

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

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

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

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

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

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

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

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

Recommended References

POLICY ON ETHICS

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

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

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

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

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

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

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

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

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

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

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

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

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**Monday, Nov. 13**

**Symposia**—8:00 a.m.

203. Neural Progenitor Cells and CNS Lineage Development  
Chair by: M.F. MEHLER  
498

204. The Neurobiology of Early Trauma: Implications for the Pathophysiology of Mood and Anxiety Disorders  
Chair by: N.H. KALIN  
498

**Special Lecture**—11:15 a.m.

205. Comparative Studies of the Organization of Neocortex  
Help Us Understand the Human Brain  
J.H. KAAS  
No Abstract

**Slide Sessions**—8:00 a.m.

206. Drugs of abuse: alcohol I  
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207. Stress: HPA axis  
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208. Beta-amyloid: secretion I  
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209. Potassium channel physiology, pharmacology and modulation I  
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210. Peptides: physiology and anatomy  
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211. Retina I  
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212. Visual psychophysics and behavior II  
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213. Ischemia: ischemic tolerance  
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214. Calcium channels: physiology, pharmacology and modulation I  
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215. Motor systems: cortex  
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680. Molecular Organization of the Postsynaptic Membrane  
**Chair by:** M.B. KENNEDY  
681. Developmental Determinants of Retinal Ganglion Cells  
**Chair by:** L.M. CHALUPA

**Special Lecture—1:00 p.m.**

682. Schizophrenia  
C.A. TAMMINGA  
No Abstract

**Special Lecture—4:15 p.m.**

683. Cell-cell Interactions Required for Synapse Formation and the Molecules That Mediate Them  
S.C. LANDIS  
No Abstract

**Slide Sessions—1:00 p.m.**

684. Postsynaptic mechanisms VI  
685. Degenerative disease: other  
686. Chemical senses  
687. Formation and specificity of synapses I  
688. Blood-brain barrier: other  
689. Visual cortex: striate IX
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Symposia—8:00 a.m.
777. Opioidergic Modulation of Long-term Potentiation in the Hippocampus: Insights Into Peptidergic Regulation of Synaptic Plasticity
   *Chaired by: C.R. BRAMHAM* .................................. 1987
778. Neural Control of Breathing
   *Chaired by: J.E. REMMERS* .................................. 1987

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780. Neurotrophic factors: receptors and cellular mechanisms V .............................................. 1989
781. Excitatory amino acid receptors XIII .............................................. 1991
782. Neuroendocrine regulation: other III .............................................. 1993
783. Ion channels: cell function III .............................................. 1995
784. Opioid receptors II .............................................. 1997
785. Cell differentiation and migration VIII .............................................. 1999
786. Neurotoxicity II .............................................. 2001
787. Alzheimer's disease: mechanisms of degeneration II .............................................. 2003
788. Long-term potentiation: physiology VII .............................................. 2005

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790. Neurotrophic factors: expression and regulation VII .............................................. 2009
791. Hormones and development III .............................................. 2013
792. Nutritional and prenatal factors .............................................. 2015
793. Neuronal death IX .............................................. 2018
794. Cerebral cortex and limbic system IV .............................................. 2021
795. Visual cortical development II .............................................. 2023
796. Transplantation: dissociated cells .............................................. 2026
797. Aging processes: molecular characteristics .............................................. 2029
798. Chloride and other channels .............................................. 2032
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800. Acetylcholine receptor muscarinic: muscarinic receptors—molecular biology and electrophysiology .............................................. 2036
801. Acetylcholine receptor muscarinic: agonist/antagonist for receptors .............................................. 2039
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**THEME B: CELL BIOLOGY**

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**Theme D: Neurotransmitters, Modulators, Transporters, and Receptors**

<p>| 34             | Acetylcholine                                                                 | Poster  | Sun AM   |          |          |          |          |
| 801           | Acetylcholine receptor muscarinic: agonist/antagonist for receptors           | Poster  |          |          |          |          | Thu AM   |
| 800           | Acetylcholine receptor muscarinic: receptors—molecular biology and electrophysiology | Poster |          |          |          |          | Thu AM   |
| 802           | Acetylcholine receptor muscarinic: receptors—expression                      | Poster  |          |          |          |          | Thu AM   |
| 11            | Acetylcholine receptor: nicotinic I                                           | Slide   | Sun AM   |          |          |          |          |
| 621           | Acetylcholine receptor: nicotinic II                                         | Poster  |          |          |          | Tue AM   |          |
| 528           | Acetylcholine receptor: nicotinic expression                                 | Poster  |          |          | Tue PM   |          |          |
| 527           | Acetylcholine receptor: nicotinic expression and treatment effects           | Poster  |          |          | Tue PM   |          |          |
| 526           | Acetylcholine receptor: nicotinic—acetylcholine—effects of nicotine on specific brain regions | Poster |          |          | Tue PM   |          |          |
| 334           | Acetylcholine receptor: nicotinic—acetylcholine—genetics: molecular          | Poster  |          |          | Mon PM   |          |          |
| 247           | Acetylcholine receptor: nicotinic—acetylcholine—pharmacology and behavior   | Poster  | Mon PM   |          |          |          |          |
| 143           | Acetylcholine receptor: nicotinic—acetylcholine—regulation, distribution, and physiology | Poster | Sun PM   |          |          |          |          |
| 142           | Acetylcholine receptor: nicotinic—biophysics                                 | Poster  | Sun PM   |          |          |          |          |
| 721           | Acetylcholine receptor: nicotinic—pharmacology                               | Poster  | Sun AM   |          |          |          | Wed PM   |
| 36            | Acetylcholine receptor: nicotinic—structure/function                          | Poster  | Sun AM   |          |          |          |          |
| 35            | Acetylcholine: distribution                                                  | Poster  | Sun AM   |          |          |          |          |
| 839           | Acetylcholine: modulators                                                    | Poster  |          |          |          |          | Thu AM   |
| 544           | Behavioral pharmacology I                                                    | Poster  |          |          |          |          |          |
| 637           | Behavioral pharmacology II                                                   | Poster  |          |          |          |          | Wed AM   |
| 305           | Behavioral pharmacology: psychostimulants                                    | Slide   | Mon PM   |          |          |          |          |
| 445           | Behavioral pharmacology: serotonin and dopamine                              | Poster  |          |          | Tue AM   |          |          |
| 632           | Catecholamine receptors: alpha adrenergic                                    | Poster  |          |          | Sun PM   |          |          |
| 149           | Catecholamine receptors: antisense and knock outs                            | Poster  |          |          |          |          | Wed AM   |
| 633           | Catecholamine receptors: beta adrenergic                                     | Poster  |          |          |          |          | Wed AM   |
| 441           | Catecholamine receptors: D, and D, pharmacology                              | Poster  |          |          | Mon AM   |          |          |
| 252           | Catecholamine receptors: D2, D3, D, pharmacology                             | Poster  |          |          | Mon AM   |          |          |</p>
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**THHEME F: SENSORY SYSTEMS**

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**4. Cellular and Molecular Mechanisms of Integration in Mammalian Retina**

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**Theme G: Motor Systems and Sensorimotor Integration**

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**Theme H: Other Systems of the CNS**

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**THEME I: NEURAL BASIS OF BEHAVIOR**

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**Theme J: Disorders of the Nervous System**

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BEHAVIORAL EFFECTS AND PENTYLENITRATOLZOLE-INDUCED SEIZURE PROTECTION OF DIZOCILPINE AND LORAZEPAM ALONE OR IN COMBINATION IN CD-1 MICE. G.A. Pizziardini, L.S. Pratt and D.J. Greenblatt. Department of Pharmacology and Experimental Therapeutics, Tulane University School of Medicine, New Orleans, LA 70112.

To evaluate the potential interaction between the NMDA receptors localized on the GABA receptor family, dizocilpine (DIZ) and lorazepam (LRZ), alone in combination, was administered in order to analyze a number of behavioral parameters as well as a pentylene nitroazole (PTZ) seizure protection assay in male CD-1 mice. Animals were injected with DIZ or vehicle and 15 min later with LRZ or vehicle. Distance traveled, rears and stereotypes were measured using an activity monitor 40 min following the first injection for 20 min (4 x 5 min). The tail vein of each mouse was then cannulated and PTZ was infused until maximal withdrawal had two tonic-clonic seizures. Behavioral effects of DIZ were observed only for the highest dose (0.1 mg/kg) and was evidenced by a significant (98%) increase in distance traveled during each of four intervals monitored. Rears and stereotypes were unaffected by DIZ at any concentration. Also, DIZ had no protective effect against PTZ-induced seizures. Lorazepam, however, significantly reduced horizontal activity, rears and stereotypes at all lower doses (0.2 mg/kg) in a dose-dependent manner. Similarly, LRZ had a dose-dependent protective effect against PTZ-induced seizures with all but the lowest concentration (0.2 mg/kg) significantly increasing the volume of PTZ needed to induce convulsions. When combined at the lowest and highest levels tested, DIZ and LRZ had effects similar to that of LRZ alone; no synergism was observed. Furthermore, the elevated horizontal activity induced by DIZ (0.1 mg/kg) was completely attenuated by LRZ (20 mg/kg).

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

The present experiments investigated effects of the selective irreversible mu opioid antagonist beta-funtrexamine (B-FNA) on discriminative stimulus and rate-altering effects of morphine sulfate (MS), the prototypic mu agonist. Male Sprague-Dawley rats were trained to discriminate among 3.2 mg/kg MS and saline under a FR15 schedule of food reinforcement. Generalization curves for MS (0.32-5.6 mg/kg) were established using a cumulative-dosing procedure. Rats were then dosed with 20 mg/kg B-FNA, by either i.c.v. or i.c.a. administration. Morphine was retested 24 h, 72 h, and 8 days after B-FNA treatment, and weekly thereafter, until recovery of the maximal stimulus effects of MS was observed. B-FNA i.c.v. produced a loss of maximal MS-like stimulus effects for up to 72 h, and also increased the dose of MS required to suppress rates by approximately six-fold. After 22 days the rate-altering potency of MS returned to control values, and MS regained the capacity to produce a maximal stimulus effect, although the dose required for MS-like responding was still greater than control values. The effects of B-FNA i.c. were significant, albeit less pronounced than those seen with i.c.a. administration, demonstrating that B-FNA is more potent when administered i.c.v. The present results suggest that B-FNA can suppress the maximal stimulus effects of MS by alkylating a sufficient proportion of the receptor pool mediating these responses. (Supported by DA03796 and K22 DA00132.)


Twelve male S-D rats were trained to discriminate between 1.25 g/kg ETHOH and saline injections, in a multi-cycle operand discrimination task. Once trained, complete dose-effect curves (DECs) were generated, once per month, using a cumulative-dose procedures (DECs 1 & 3: 0.25, 0.75, 1.25, 1.75, 2.25 g/kg or DEC 4: 0.4, 0.625, 1.25, 1.875, 2.25 g/kg). The same doses were tested singly in acute testing sessions over the cumulativebernagBei DECs were generated within each cumulative procedure (DEC1 vs DEC3), (DEC3 vs DEC4). n.s.; DEC2 vs DEC4 (P<11.13); n.s., however, significant differences were observed between the two testing procedures (ED50s: DEC1 vs DEC3: 1.06 ± .25 vs 1.06 ± .22; DEC2 vs DEC4: .74 ± .15 vs 2.2 ± .17). Tests of the 1.25 g/kg ETHOH training dose engaged 100% ETHO-appropriate responding under acute testing procedures but only 76% ETHO-appropriate responding under cumulative testing procedures. When blood alcohol concentrations (BACs) were quantified by GC analyses, cumulative testing procedures always produced significantly lower BACs than acute testing procedures (n=8, p<.001). Group mean BACs produced by the 1.25 g/kg ETHOH training dose were 70 ± 6 mg/dl and 126 ± 5 mg/dl when tested in cumulative and acute testing procedures, respectively. Rate functions did not differ between either cumulative procedures or between cumulative and acute procedures. These data may suggest that different measures, such as cumulative vs acute, of ETHO-appropriate responding may play an important role in the metabolic processes (absorption or distribution) may influence the behavioral choice and blood alcohol concentration DECs in rats.

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

Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions.

Cyclophosphamide is a commonly used chemotherapy agent for breast cancer and induces emesis in the ferret. In order to examine the emetogenic effect of cyclophosphamide, ferrets (1.0-1.8 kg) were placed under general anesthesia (isoflurane 5%-O2 mixture) and were administered logarithmic doses of i.v cyclophosphamide. The mean number (+SE) of emetic episodes between 12.0 ± 0.9 and 2.84 ± 1.9 retches at 56 mg/kg, 7.3 ± 3.2 and 30 ± 5.17 ± 1.00 mg/kg, 23.3 ± 0.9 and 85 ± 24.0 at 177 mg/kg, and 23.5 ± 7.1 and 62.5 ± 3.8 at 237 mg/kg. In addition, various antietemics were given i.v. immediately following cyclophosphamide injection. Ondansetron reduced emetic episodes by 0% and 43% (0.04 and 0.07 mg/kg), metoclopramide by 65% and 98% (4.08 and 7.07 mg/kg), and droperidol by 16% and 24% (0.45 and 0.25 mg/kg). These results indicate that cyclophosphamide induces emesis in a dose-dependent manner and may be useful in evaluating conventional and complementary therapies for the treatment of chemotherapeutically-induced emesis. Support provided by the U.S. Army Breast Cancer Research Program #DAMD17-94-J-44252.


This laboratory recently reported that behavioral effects of (-)-amphetamine can produce the effect-additivity of stimulus elements of compound drug stimuli (FBD&K, 21:1017-1023; Psychopharm 110:309-319; J Psychopharm 7:52-59). With respect to stimulus control by compound stimuli, Fantino & Logan have suggested that the specific stimulus element or dimension which controls the behavioral operand is largely beyond experimental control and may even be idiosynratic (1979). The experimental analysis of behavior: a biological perspective. San Francisco: W.H. Freeman. Using a two-choice drug discrimination task, we were trained with amphetamine as a stimulus to saline as a training stimulus. We used a single subject analysis strategy to examine which specific elements of the compound morphine cue controlled behavioral choices. Cross-generalization profiles were determined for a number of over-the-counter (OTC) medications which were hypothesized to engender subjective elements of a compound morphine cue including sedation, lethargy, and analgesia: decongestant, antihistamines, anticholinergics, and ophthalmics. When tested in combination with saline, none of the OTC compounds produced a greater percentage of drug-appropriate responding >50%. Based on an individual rats’ cross-generalization profile, each subject was tested for ‘stimulus-element-additivity’ by administering and testing variations of binary and ternary combinations of these same OTC compounds. A test combination was formulated from the addition of single TC drug stimulus elements for each rat which engendered >95% morphine-appropriate responding. These test combinations appeared to be idiosynratic, drug-dependent, and to follow rules predicted by simple effect-additivity.


The dose of a drug used for training can influence the characteristics of drug discrimination behaviour established with single drugs. However, whether this also applies to discriminations based on mixtures of drugs, three groups of rats (n=10 each) were trained to discriminate mixtures of (+)amphetamine (0.2-0.8 mg/kg) plus pentobarbitone (5-20 mg/kg) from saline in a standard discrimination procedure in the presence of food reinforcement; the ratio between the training doses was constant at 1:25 for each group. The groups acquired the discriminations to an accuracy of 90-97% and, in each case, generalisation to complete drug was clear and dose-related, but less than complete in most experiments (68-86%). There was also partial generalization when either apomorphine (50%) or nicotine (63%) was administered alone, but only when one of the elements of the mixture in rats trained with the larger doses of the mixture, neither apomorphine nor nicotine increased responding above vehicle levels. Doses of pentobarbitone that were half of those used in training produced little or no discriminative response when administered alone to the rats trained with the two smallest doses of the mixture; the same doses of pentobarbitone increased responses to amphetamine or apomorphine in a supra-additive manner. Strikingly, some doses of apomorphine and pentobarbitone that alone produced vehicle-like responding (11%) produced almost complete generalization (80%) in rats trained with 0.4 mg/kg of amphetamine plus 10 mg/kg of pentobarbitone; this interaction was not seen in the rats trained with either smaller or larger doses of the mixture. The complex pattern of results suggests that doses of drugs used for training play an important role in the discrimination of abused drug mixtures, but no simple rules to predict the influence of training dose have been ascertained (supported by NIDA grant DA 05543).
368.1
THE PATTERN OF CELLULAR ACTIVATION SEEN IN RESPONSE TO ACUTE RESTRAINT STRESS IN HYPOTHALAMIC AND HYPOTHALAMIC-AMINGEAL NEUROGENIC STRESS MODELS: Y. Viar* and P.E. Sawchenko.
The Salk Institute, La Jolla, CA 92037.
Acute restraint reliably activates the hypothalamo-pituitary-adrenal (HPA) axis, and is among the commonly employed stress models. To provide an overview of the CNS systems that are activated in response to this challenge, we followed the time course of Fos protein expression induced in rat brain by a single 30 min restraint session. The temporal patterns of cellular activation differed greatly among stressors. For example, we observed much more robust activations of Fos in animals sacrificed 1-2 hr after stress, and more toward front levels by 4 hr. Cell groups displaying Fos induction included the paraventricular nucleus of the hypothalamus (PVH), where responsive neurons were localized primarily in the hypothalamic zone of paraventricular organizer, with secondary accumulations in autonomic-related subdivisions and in the magnocellular parvocellular systems. Beyond the hypothalamus, Fos-containing neurons could be grouped as belonging to three broadly defined systems. Induction was prominent in the limbic region of the telencephalon, including the infra-and prelimbic cortices, the lateral septal nucleus, bed nucleus of the stria terminalis, and the basolateral and medial amygdaloid nuclei. A second major focus comprised cell groups involved in the processing of somatosensory and/or nociceptive information; such structures included the dorsal column nuclei, the peri-aqueductal gray, cholinergic cell groups of the magnocellular tegmentum, and the midline and intralaminar nuclear complexes of the thalamus. Finally, brainstem catecholaminergic cell groups readily displayed robust restraint-induced Fos expression.

Prominent among these were the locus coeruleus, and cells in the medial part of the nucleus of the solituary tract, as well as amineergic regions of the rostral and caudal ventrolateral medulla. This general pattern of restraint-induced cellular activation is strikingly similar to that which has been described following footshock or immobilization, and contrasts starkly with profiles seen in response to systemic stresses such as hemorrhage or immune challenge. While the categorization of certain stress models as "neurogenic" appears to have some basis in induced patterns of immediate-early gene expression, it remains to be determined whether they activate the central limb of the HPA axis through common circuitry and mechanisms.

368.2
SEQUENCE OF STRESS-INDUCED ALTERATIONS IN INDICES OF CELLULAR ACTIVATION IN PARVOCOULEAR NEUROSECRETORY NEURONS: MARKERS VERSUS MECHANISMS. K.J. Kovacs* and P.E. Sawchenko.
The Salk Institute, La Jolla, CA and Inst. Exp. Med., Budapest, Hungary.
Acute ether stress results in increased ACTH and corticosterone secretion that peak at 5 and 30 min, respectively, after the challenge. Using cDNA probes to intronic sequences of genes encoding ACTH secretagogues in parvocellular neurosecretory neurons of the paraventricular nucleus (PVH), we have found these events to be accompanied by rapid and transient transcriptional activation of cAMP-responsive factor (CRF) and βT mRNA expression (peak at 5-15 min), and a delayed up-regulation of arginine vasopressin (AVP) mRNA (90-120 min). To gain insight into possible molecular mechanisms regulating stress-induced CRF and AVP expression in vivo, we have compared the time course of other stress-induced activation of representatives of three transcription factor classes: immediate-early genes (c-fos and NGF-B), a POU domain factor (Brn-2), and the cAMP response element binding protein (CREB), using antisera specific to its transcriptionally active, phosphorylated, form (P-CREB). Functional DNA binding sites for both Brn-2 and P-CREB have been identified on the CRF promoter. In response to ether stress, c-fos and NGF-B mRNA induction in the PVH were maximal at 30-60 min, and that of Fos protein peaked at 90-120 min. Brn-2 mRNA was found to be constitutively expressed in parvocellular and magnocellular compartments of the PVH, and its expression did not change over the 4 hr observation period we employed. P-CREB was induced in parvocellular neurons with a time course which paralleled that of CRF mRNA expression, reaching a maximum 5-15 min after exposure to ether vapor. In vivo inhibition of protein synthesis by cycloheximide prior to stress resulted in a marked reduction of the Fos protein response to ether in the paraventricular PVH, and attenuated the stress-induced rise in AVP mRNA. Cycloheximide treatment did not, however, alter significantly P-CREB induction or stress-induced CRF mRNA expression. The stress-induced transcriptional activation of the AVP and CRF genes in hypophyseotropic neurons appears to involve independent mechanisms, that respectively do and do not require de novo protein synthesis.

368.3
Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404 and Dept Neurosciences and Cell Biology UMDNJ-RW Johnson Medical School, Piscataway, NJ 08854.

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

368.4
Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404.

Previous research from this laboratory suggests that nicotine (NIC) elicits ACTH secretion by activating brainstem catecholamine neurons projecting to the paraventricular nucleus of the hypothalamus. The present study further examined this hypothesis. In experiment 1, rats received iv injections of vehicle, 0.045, 0.09, or 0.18 mg/kg NIC at a rate of 0.09 mg/kg/min and were sacrificed 1 hr later. Immunohistochemical analysis showed that NIC dose-dependently induced c-Fos expression throughout the NTS-A/C. This expression was a linear function of dose from 0.045-0.135 mg/kg, with the 0.18 mg/kg dose causing a far greater induction. In experiment 2, rats received vehicle or 4 μg of the nicotinic antagonist mecamylamine (MEC) into the fourth ventricle 15 min before iv administration of 0.09 mg/kg NIC. The results showed that only a small fraction of the NTS-A/C neurons expressed NIC-induced c-Fos also contained the catecholamine synthesis enzyme tyrosine hydroxylase. However, MEC antagonized NIC-induced c-Fos expression throughout the NTS-A/C. The present results are consistent with the hypothesis that NIC elicits ACTH secretion via activation of brainstem neurons, some of which are catecholaminergic. (Supported by DA03977)
638.5
INDUCTION OF THE IMMEDIATE-EARLY GENES c-fos in THE ADRENAL AND LH SYSTEMS FOLLOWING LOUD NOISE STRESS, S. Campeau*, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The paraventricular nucleus of the hypothalamus is commonly thought to be the final common relay essential for stress-induced glucocorticoid release. However, the anatomical pathway(s) through which neurogenic stressors activate the paraventricular nuclei are not well characterized. In an attempt to determine more precisely how the paraventricular nuclei is activated by stress, a loud noise stimulus was used. By 30 min post-exposure, c-fos mRNA was used to map the pattern of neuronal activation in the paraventricular nuclei. Induction of c-fos mRNA was used to map the pattern of neuronal activation in the paraventricular nucleus. To evaluate neuronal activation, c-fos mRNA was measured in multiple forebrain and limbic areas as well as throughout the auditory system (from the cochlear nuclei to the auditory cortex). The 30 min period of repeated loud noise and limbic stress that resulted from following swim and restraint stress (Cutinan et al., Neurosci. 64:477, 1995). Lesion studies are underway to determine which of the auditory relay nuclei provide the starting point of a mono-synaptic pathway ultimately innervating the paraventricular nucleus of the hypothalamus.

638.7
TIME COURSE OF FOS STAINING FOLLOWING EXPOSURE TO RESTRAINT IN CHRONIC, INTERMITTENTLY COLD STRESSED ANIMALS. S. Sharman* and M.F. Dallman, Dept. of Physiology, UCSF, San Francisco, CA 94134-0444.

Exposure of chronically stressed animals to a novel stress resulted in similar or enhanced hypothalamic-pituitary-adrenal (HPA) responses to those in control animals despite the persistent negative feedback signal due to the chronic stress. It has been suggested that prior chronic stress produces a facilitatory trace that balances or overcomes the negative feedback effects of chronic stress, although the neuroanatomical sites subserving this facilitation are not yet known. We have shown that animals exposed to chronic, intermittent cold stress (4 h a day at 4C) exhibit enhanced HPA responses to a 30 min period of restraint compared to undisturbed controls (C1). In the present study, we sought to determine which sites may underlie this chronic stress-induced facilitation by immunohistochemistry for the fos protein. C1 and C2 animals were perfused under basal conditions, at 15 and 30 min restraint and at 30 min following termination of restraint. Preliminary data indicate that fos staining peaked in the paraventricular nucleus of the hypothalamus at 30 min post-exposure in both C1 and C2 animals, with no differences between groups in number of fos-stained cells at any time examined. Present data also indicate that staining in the arcuate nucleus is lower in C1 than in C2 animals at 30 min. Ongoing work is examining fos staining in other areas of the hypothalamus and brainstem with the goal of characterizing the neuroanatomical sites that may underlie chronic stress-induced facilitation of HPA responses to a novel stressor.

638.9

Adrenalectomy (ADX) has been shown to enhance neuronal activity in the paraventricular nucleus of the hypothalamus (PVN) as measured by expression of c-fos-like immunoreactivity (Fos-LI) as well as fos-mediated gene expression. In the present study, were the BMNS and pathogen challenge on PVN and adrenergic system. In the present study, we examined twenty-five intact or ADX Sprague-Dawley rats were injected with either phospholipopolysaccharide (LPS) (40 mg/kg) p.o. or saline (n=13 per group) and Fos-LI measured at 2 or 5 hrs before perfusion. Experimental variables were: ADX duration, short term (≤14 days) or long term (≥21 days); and pre-perfusion challenge, control (PBS) or pathogen (LPS) performed using a fos-antiserum (Cambridge Biochem. Inc.) and solutions obtained from Veestman elite Sheep (116). Immunohistochemistry was counted using image analysis. Results confirm short term ADX non-challenged animals have significantly more Fos-LI in the PVN compared to the control animals. However, expression induction of ADX had been increase in PVN and LPS at 2 hrs but not at 5 hrs. The main factors, ADX and LPS appeared additive. LPS elicited greater effect on neuronal activity in the PVN in long term ADX (14 days) versus short term ADX (3 days). The same was particularly true at low doses of LPS, where the baseline levels of Fos-LI in the paraventricular PVN was significantly lower (p<0.05) in short term ADX compared to long term ADX. However, the dose of LPS at 0.05 mg/kg (a 50 fold lower) induced a significant increase in Fos-LI. The dose of LPS at 0.05 mg/kg (a 50 fold lower) induced a significant increase in Fos-LI. The dose of LPS at 0.05 mg/kg (a 50 fold lower) induced a significant increase in Fos-LI. The dose of LPS at 0.05 mg/kg (a 50 fold lower) induced a significant increase in Fos-LI.

638.10

Neurotensin (NT) is a poorly understood neuropeptide that is known to modulate a wide range of neuroendocrine functions in the hypothalamus. We are currently investigating the effects of chronic administration of NT on hypothalamic-pituitary-adrenal (HPA) functions, in order to determine whether chronic administration of NT modulates HPA activity and behavior. We administered NT (1-10 nmol/rat, 14 days/week) intracerebroventricularly (icv) or intracranial (iac) to rats and recorded plasma adrenocorticotropin (ACTH) and corticosterone (CORT) levels. We found that chronic icv administration of NT (10 nmol/rat, 14 days/week) significantly increased plasma ACTH levels and reduced corticosterone levels. These results suggest that the observed c-fos response to KM108 is T-dependent. Supported by Can. MRC.

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638.11

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

During the first 2 weeks of life, rats exhibit low basal and stress-induced corticosterone (B) secretion and blunted adrenal sensitivity to ACTH. Maternal separation for 24h (SEP) increases basal adrenocortical activity, potentiates stress responses and most likely results in sympathetic activation. However, how increased catecholamine secretion following SEP could participate in the enhanced ACTH secretion is unknown. Before testing the effects of adrenergic blockade on ACTH secretion following SEP, we first used a 3min exposure to ether as an acute stressor known to stimulate catecholamine release. The effects of propranolol (3mg/kg,ip-60min), prazosin (0.4, 2 mg/kg,ip-60min) or idazoxan (1.5 mg/kg,ip-30min) pretreatment on basal and ether-induced ACTH and B secretion were determined in 8-10 days-old (d) and in 22-23d intact neonates. Saline pretreatment was used as a control. Blood samples were collected at 0, 5, and 30min after the onset of ether stress. Both prazosin and idazoxan increased basal and stimulated ACTH and B secretion at the highest doses in 10d neonates compared to saline-treated controls. Propranolol had no effect on ACTH or B release. In contrast, in 22d neonates, propranolol and prazosin decreased stress-induced ACTH secretion in an adult-like fashion while idazoxan tended to increase basal ACTH secretion. These results suggest that the control of neonatal ACTH secretion by alpha1-adrenergic receptors shifts from being inhibitory to mainly stimulatory with age. Alternatively, age-related changes in the distribution of hypothalamic adrenergic receptor types or efficacy of coupling to second messengers might dictate different effects on ACTH secretion in early neonatal life. We are now determining whether changes in central catecholamine release and/or alpha1-adrenergic receptors would affect the control of ACTH release following SEP in neonates. (Funded by NSERC and FCAR of Canada).

638.12

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

Lactation in the rat is associated with elevated corticosterone (B) release and blunted adrenocortical responses to stress. Because B secretion in the forced swim test (FST) at the time of the first swim (15min) is thought to facilitate immobility retention in a second session (5 min) 24h later, we determined whether lactating females would exhibit changes in immobility related to the magnitude of their basal and stress-induced ACTH and B secretion. Females in early (EL, db-10) or late (L, d17-18) lactation were compared to virgins or male rats for their acquisition and retention of immobility as well as for their ACTH and B responses to the FST. Intact lactating and virgin females were compared to their adrenal-removed (ADX, 5 days) or ovariolectomized (OVX, 10 days) counterparts. Although ACTH and B responses to stress were similar in males and virgin females, immobility scores were greater in males than in virgins on both days of testing. Immobility retention occurred in young males, but was not observed in any of the female groups, lactating or not. In contrast, basal B secretion was elevated in lactating females (EL>L>V), the magnitude of the ACTH response was blunted in intact and ADX lactating females compared to virgins (V>EL>L), but no differences in immobility scores were observed as a function of lactation. In OVX females (V or EL) immobility was decreased and basal ACTH and B was slightly reduced compared to intact rats. We conclude that: 1) dissociation between endocrine and behavioral responses occur as a function of sex and lactational status, questioning the facilitatory effect of B on immobility retention and 2) blunted stress responses in lactating females is not caused by increased basal B and thus enhanced sensitivity or facilitation of the adrenocortical system since they are still observed in ADX females. (Funded by MRC Canada).

638.13

WITHDRAWN

638.14


The neuro-endocrine protein "VGF" contains multiple potential cleavage sites and a cleavage-amidation site. Antiserum to five VGF domains, plus the putative amided sequence (NH₂)- were used in immunocytochemistry and Western blot. In Guinea-pig, bovine, pig and human (n=8-15/species) all sera stained anterior pituitary endocrine cells. As in rat, immunoreactivity for the VGF domains was mainly found in lactotropes and/or gonadotropes, although incomplete overlap and inter-species differences were seen. Large, intermediate and small molecular weight forms were found in Western blot with most sera. While *NH₂-immunoreactivity was restricted to intermediated small forms in rat, no reactivity was found for the *NH₂-serum, in either immunocytochemistry or Western blot.

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

638.15

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

Glucocorticoids controlling adrenergic medullary secretion are known to be centrally located since administration of glucocorticoids directly into the brain elicits a sympathoadrenergic response. In addition, systemic glucocorticoid elicits sympathoadrenergic response in desaminase deficient rats. However, these effects are not seen in that hindbrain. The present study mapped the hindbrain between the facial nerve and obex of rats (n=70) for glucocorticoid sensitive sites. The antimetabolic glucose analogue, 5-glucose (STG, 12-24 ug in 100-200 ml) and 0.9% saline were injected through small diameter cannulas. Blood was collected at 8 intervals during a 156 min period beginning 15 before the injection. At positive sites, blood glucose began to rise with short latency and peaked at about 60 min post injection. Positive sites were found to be closely associated with catecholamine (CA) cell groups, especially A1/C1 and C2, where 24 ug of STG raised glucose levels from 92 to 240 mg/dl. Surrounding sites, including ventricular sites, were less positive or negative. These hindbrain CA neurons may be involved in the adrenergic medullary hyperglycemic response, since they express Fos-like immunoreactivity in response to glucocorticoids and those of A1/C1 directly innervate renal nerves. Our findings suggest that these additional experiments will be required to determine whether A1/C1 and C2 neurons are themselves glucocorticoster or whether other neurons in close proximity to these CA neurons are the putative glucocorticoster.
639.3

In the present study, we demonstrated that psychosensational factors significantly altered the response of the transplantable adenocarcinoma (SC115) to adriamycin (AD) or cyclophosphamide (CY). Male DDS mice were either reared in a clean environment (CG) or reared in a dirty environment (DG) and then exposed to SC115 following tumor cell or vehicle injection. Chemotherapy or drug vehicle injections (3 times in 7 day intervals) were started when tumors reached 1 g. To monitor the toxic effects of chemotherapy, control mice bearing no tumors (NCTC) also received 3 chemotherapy treatments beginning 16 days following tumor vehicle injection. Survival probability was significantly increased in mice in IG (47%) compared to GI (19%) groups due to greater inhibition of tumor growth. The number of deaths due to the toxicity of chemotherapy was similar between groups. This animal model should aid in elucidating mechanisms that may govern the responsiveness of tumors to chemotherapy and the variability of chemotherapeutic efficacy in human cancer patients.

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

639.4

The effects of pertussis toxin (PTX) pretreatment on basal and morphine-affected changes of tuberoinfundibular dopaminergic (TIDA) neuron activity and serum prolactin level in ovariohysterectomized Sprague-Dawley rats treated with a long-acting estrogen (polycyclodextrinophosphate, 0.1 mg/rat, sc) were assessed in the study here. The activity of TIDA neurons was determined by measuring the turnover rate of dopamine, the concentrations of DopAC or the accumulation of DopA in the median eminence. Acute (30-90 min) treatment of PTX (0.025-2.5 µg/rat, ivc) had no significant effect on any of the parameters measured. Prolonged (24 h) treatment of PTX (1 µg/rat, ivc), while had no effect on basal TIDA neuron activity either, significantly reduced morphine’s (10 mg/kg, sc) inhibitory effect on TIDA neuron activity (using DopA, but not DopAC as the index), and stimulatory effect on PRL release. The dopamine levels in the median eminence, however, were significantly reduced by prolonged treatment of PTX, indicating a reduction of TIDA neurons. These results suggest that PTX-sensitive GTP-binding proteins may be responsible for the maintenance of TIDA neurons, and for mediation of the inhibitory effect of morphine on TIDA neuron activity, and in turn, the stimulation of prolactin secretion.
639.7


The intervation of the footpath has been utilised recently as a model for the chemical coding of defined autonomic pathways. The aim of this study is to use the trans-synaptic retrograde transfer of pseudorabies virus (PRV) to map the distribution of chains of connected neurons directed to sudomotor and vasomotor endpoints in the footpath. We used young New Zealand white rabbits to show the distribution in the footpath with sodium pentobarbitone (60 mg/kg ip) and 0.5 µl of a solution containing PRV was injected into several sites in the left forepaw. After survival periods ranging between 24 and 100 hours, rats were anaesthetised and perfused transcardially with 4% paraformaldehyde. Frozen sections were cut and assayed against PRV or its main protein (TH) and a range of peptides. Infected neurons were first detected in the ipsilateral stellate ganglion 40 hrs. after inoculation. Infected neurons included populations which were TH-positive and TH-negative (cholinergic, approx. 15%). Over the next 50 hrs. infected neurons were found first in the sympathetic preganglionic cell groups of the upper thoracic spinal cord then correspondingly in the rostral paraventricular medulla and paraventricular paracervical nucleus (PVN). Up to 50% of the neurons in the medulla were catecholaminergic whereas infected neurons in the PVN were not vasopressin positive and less than 1% were colocalised with oxytocin. Subsequently neurons were infected in the lumbar terminals. bed nuclei of the stria terminals and pargiotic area. Despite a general codistribution of the latter neurons with neurons containing atrial natriuretic peptide (ANP) none were found to be ANP-positive. These results demonstrate that both the sciatic and the posterior tibial nerve affects the footpath and provide the basis for nonchemical coding of these pathways.

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

639.9


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

639.11


We have shown in previous studies that the activity of sympathetic and parasympathetic nerves in the cervical region affects parathyroid hormone (PTH) and calcitonin secretion. Additionally, cholinergic neurotransmission in superior cervical ganglion (a site of origin of the sympathetic innervation to the thyroid parathyroid complex) was significantly modulated by both hormones. To further substantiate the hypothesis of the existence of a feedback loop in neural regulation of calcium homeostatic hormones, the effect of PTH and calcitonin on acetylcholine uptake and on the choline synthesis in neurons of the cervical sympathetic nerves was measured. To this end, the cervical sympathetic nerves were isolated from the gland, perfused with oxygenated artificial cerebrospinal fluid containing PTH or calcitonin and the uptake of tritiated choline was measured. The uptake of the tracer was found to be significantly decreased by PTH (20% at 100 ng/ml), but increased by calcitonin (20% at 100 ng/ml). By RIA, the secretion of PTH and calcitonin were found to be significantly suppressed by the respective hormones. These results suggest that both hormones modulate the activity of the cervical sympathetic nerves in the cervical region.
639.13

In order to study the sympathetic-adrenal interactions of the adrenal cortex, we have established cultures of adrenocortical and sympathetic ganglion cells. Sympathetic neurons from sympathetic chain ganglia of 4-6 day old rat pups are grown in supplemented DMEM/F12 medium and dissociated adrenocortical (AC) cells from adult rats at various times. We have adapted immunocytochemistry (ICC) to characterize the cell cultures and to study the relationships between the sympathetic neurons and AC cells. The sympathetic neurons usually cluster in groups of 2-10, and begin to extend neurites within 24 hours. Neurite length can exceed 5 mm, and neurites often form fascicles after 4 days. These sympathetic neurons, identified by 2H3 ICC, continue to express tyrosine hydroxylase and dopamine-β-hydroxylase. A subpopulation are also immunoreactive for VIP. We have used sympathopenesis as a marker for neuronal maturation and potential for functional autonomic synapse formation. Synaptophysin is detectable after 2 days, and forms puncta along neurites and cell bodies after 4 days, suggesting the formation of presynaptic release sites. AC cells are generally rounded, contain many lipid droplets, express 3-β-hydroxysteroid dehydrogenase, and secrete steroids. The addition of AC cells to sympathetic cultures at days 2, 7, and 14 does not appear to affect neurite extension or the expression of 2H3, dopamine-β-hydroxylase, tyrosine hydroxylase, or synaptophysin in the sympathetic neurons. The neurites grow close to, over, and around the AC cells. Thus, these cultures of the AC cells are not their secreted products, for the study of the sympathetic control of AC cellular functions.

639.15
DRINKING AND BLOOD PRESSURE RESPONSES TO CENTRAL INJECTION OF L-NOSINE IN CONSCIOUS RATS. H. Liu, M.L. Terrell, J.Y. Poirier, A. Kedden*. Deaconess Hospital, Galveston, TX and Department of Pharmacology, Hershey Medical Center, Pennsylvania State University, Hershey, PA.

We studied the effect of NOS inhibitor l-arginine methyl ester (L-NNAME), a potent blocker of nitric oxide (NO) synthase, on water intake and blood pressure (BP) responses in conscious male albino rats during osmotic stimulation and hemorrhage. Thirty min after injection of 1.0 M NaCl (15 mg/kg, s.c.) or 10 min after the beginning of hemorrhage (0.7 ml/min to a 20% body volume loss), artificial cerebrospinal fluid (cSF) or L-NNAME (10, 250 or 500μg/p) were injected ivc. Ten min later, water was presented to rats and its cumulative intake was measured for 2h. During osmotic stimulation and hypovolaemia L-NNAME at doses of 250 and 500μg/p but not 10μg, attenuated (p<0.05) water intake compared to the control group. Mean arterial BP, which increased 30 min after osmotic stimulation (p=0.05), remained at a lower level after treatment with cSF and L-NNAME, but decreased progressively below basal levels after treatment with cSF and the lowest dose of L-NNAME (10μg). Ten min after starting hemorrhage, BP fell ~ 60 mm Hg (p<0.05) in all groups but increased after hemorrhage ceased. The fall in BP associated with hemorrhage returned to control levels after treatment with 250 and 500μg/p of L-NNAME but not after cSF or 10μg of L-NNAME. These results indicate that NO is involved in the regulation of drinking behavior and may have an important role in the control of BP during osmotic stimulation and hypotensive hemorrhage. (Supported by NIH grants NS41-2305 to MK and HDRO1-25498 to JYSL.)

639.17
Spatial organization and representation are impaired in Cushing’s disease. H. Forger*, H. Cohen*, M. Somma*, and A. Lacroix*. Laboratoire de neurosciences de la cognition, Université du Québec, Montreál, Qc, H3C 3P8, Canada; 2 Service d’Endocrinologie, Hôpital Notre-Dame; et Service d’Endocrinologie, Hôpital Hôtel-Dieu, Université de Montreál, Qc, H2L 4K8, Canada.

It is now well established that the brain is a major target for glucocorticoid action and emotion are affected by inadequate levels of circulating hormones. The hippocampus is a crucial structure as it is the richest in glucocorticoid-binding receptors and is thus particularly vulnerable to glucocorticoid excess. In this study, we tested whether an excess of glucocorticoid hormones has an effect on the treatment of spatial organization and representation. In the animal literature, the hippocampus was found to be dependent on hippocampal integrity. In this perspective, we compared the spatial organization and representation (line orientation, visual organisation, visual recognition, Block Design, Object assembly, Picture completion) of 12 patients with chronic hypercortisolemia due to Cushing’s disease (CD) to that of healthy control subjects yoked for age, sex and education (mean ages = 45.2±9.4 vs 45.2±9.3 years). MANOVA results showed a general impairment in spatial tasks (Pillais: .551, F(12.359, 3.27) = .027) and, except for visual recognition, subsequent univariate F-tests showed group differences in all tasks (all p’s < .05). These results show that impairment in the treatment of spatial information may be associated with elevated cortisol levels in patients with CD. It remains to be established whether the observed deficits in the treatment of spatial information, in CD patients, occur at a perceptual or at a processing/treatment level.

639.14

Recent studies show that adrenocortical cell secretion is modulated by adrenergic modulatory cells, and hence, indirectly by spinal nerve activity. We have chosen the frog adrenal as a model to study the chromaffin cell modulation of adrenocortical secretion because chromaffin cells are intrinsically metabolically independent. For this reason, frog chromaffin cells, like a subpopulation of their mammalian counterparts, extend processes which might allow direct interactions between corticosteroidogenic and chromaffin cells. To facilitate the investigation of adrenocortical interactions, we have established and characterized primary cell co-cultures of frog adrenal cells. The corticosteroidogenic cells have a low basal steroid secretion rate (1.4±0.7 ng/cell/24hr) but when co-cultured with the immunocytochemical release of adrenaline. The chromaffin cells continue to express tyrosine hydroxylase, PMSMT, dopamine β-hydroxylase, and enkephalin and are also immunoreactive for NCAM and linc (amphibian neural cell marker). Techniques for demonstrating neurotransmitter release from chromaffin cells elicited by 10 minute stimulation with 2 mM carbachol or 55 mM potassium. Activation of adrenocortical cells following 30 minute stimulation with 2 mM carbachol or 10M ACTH is shown by Fos. These techniques will be used to test the hypothesis that chromaffin cell activity directly modulates steroidogenesis.

639.16
FLAVONES ANTAGONIZE LIPOPOLYSACCHARIDE-INDUCED SICKNESS IN MICE. R J Fiskin, R Corbet* and J T Winstin.


Previous studies indicate that indomethacin, methyldprednisolone and an interleukin-1 receptor antagonist inhibit lipopolysaccharide (LPS)-induced sickness behavior in rats. In the current studies we replicated and extended these findings in the mouse to a novel class of anti-inflammatory drugs: flavones. A relatively low dose of LPS (15 μg/kg, ip) consistently induced a 50% decrease in the total amount of activity in the social interaction test of a juvenile conspecific by an adult male mouse. Pretreatment with methylprednisolone (30 mg/kg, ip), indomethacin (3-10 mg/kg, ip), and ibuprofen (10 mg/kg, ip) but not tidzapril (100 mg/kg, ip) 1 hr before LPS injection completely blocked LPS-induced sickness behavior. Dexamethasone (0.1 mg/kg, ip) significantly attenuated LPS-induced sickness behavior. The flavones LBZ-05/7 (0.25 mg/kg, ip), L86-8276 (0.03 mg/kg, ip) and chrysin (10 mg/kg, ip) also antagonized LPS-induced sickness. The psycho-motor stimulant amphetamine (2-4 mg/kg, ip) did not relieve, and actually exacerbated LPS-induced decreases in investigation. This model may provide a sensitive method of identifying compounds which selectively antagonize behavioral effects associated with interleukin release.

639.18

Dopamine (DA) neurons and their terminals are widely distributed within the hypothalamus and play an important role in the regulation of the hypothalamic neuroendocrine system. In our previous work, DA (30 μM) was found to decrease glutamate neurotransmission. This DA effect was specifically due to a decrease in the amplitude and frequency of exocytotic putaway synaptic potentials (EPSPs), and could also be due to a DA-mediated increase in GABAergic neurotransmission. Activity from GABAergic neurons that activation of GABAergic neurons might be one of the mechanisms of DA's inhibitory effect on glutamate activity we used whole cell patch clamp recordings to investigate this hypothesis. In current clamped neurons (average membrane potential -60 mV), DA application (10-200 μM) decreased the frequency of spontaneous GABA- mediated IPSPs in the majority of the tested cells (10 of 11). In cells held at -25 mV, DA (10 μM) evoked the appearance of inhibitory postsynaptic currents (IPSCs) in 5 of 5 cells and significantly increased the amplitude (3 of 11 cells; by 150%) and frequency (9 of 11; 300%) of spontaneous IPSCs. The spontaneous and DA-evoked IPSCs were blocked by 50 μM bicuculline, verifying their GABAAergic origin. This finding together with data showing that DA in this pituitary gland system that DA has acted at least partly achieved through the potentiation of GABA release.
CALCIUM REGULATION OF HYPOTHALAMIC FOS GENE EXPRESSION FOLLOWING DEPOLARIZATION D.M. Won* and H.S. Gainer, Lab. of Neurochemistry, NIMH, NIH Bethesda, MD 20892.

The complexities of the in vivo environment in the brain make it difficult to determine the signal transduction mechanisms involved in stimuli-coupled neuropeptide gene expression. Therefore, we have developed an in vivo slice explant model for examining effects of neurotransmitter/neurosteroid stimulation of neurons in a controlled environment. Using electrophysiological and morphological techniques, acute slice explants were used to study Fos expression in the paraventricular nucleus of the hypothalamus (PVN) derived from postnatal rats. In 1.5 hrs of stimulation in vitro, either K+ (40 mM), glutamate (100 μM) or veratidine (1 mM) depolarized the pituitary, and topographically distinct patterns of Fos expression in magnocellular, parvocellular and periventricular regions of the PVN as well as the supraopticohypophyseal complex (Neuroendocrinology, 1994). The role of calcium in Fos gene expression, using the above depolarization paradigms, was examined either in the absence of extracellular calcium or by permeating the acute slice explants with N-channel or L-channel calcium blockers. While the absence of extracellular calcium, Fos induction did not occur after depolarization. The L-channel dihydropyridine (Nifedipine, 1 μM) was most effective in inhibiting depolarization-induced Fos expression. In contrast, the N-channel blocker (ω-conotoxin, 1 μM) had little or no effect on Fos expression. These data strongly implicate calcium influx, via L-channels, as the principal mechanism of depolarization-induced Fos gene expression in neurons in the mammalian hypothalamus and suggest that this experimental preparation will be very useful in exploring the regulation of other immediate early genes, and neurotransmitter/neuromodulatory influences.

GASTROINTESTINAL REGULATION: GASTROESOPHAGEAL CONTROL

640.1


The efferent fibers from magnocellular and parvocellular subunits of the PVN have been known to subserve separate roles in posterior pituitary and brainstem nuclei supplying the vagus nerve (neuroendocrine and autonomic functions, respectively). To investigate the neurons projecting to both neuroendocrine and autonomic nervous systems especially projecting to the stomach, viral and CH-FRP retrograde transneuronal labeling methods were used. Pseudorabies virus(PRIV) was injected into the stomach wall of the adult Sprague-Dawley rats. After 2 days, the rats were given another injection of CH-FRP into the posterior pituitary with the aid of stereotaxically selected. After 5–7 days survival, rats were perfused with 4% PLP and their brains were processed for light detection of CH-FRP, CT and oxytocin. In longer surviving groups, some cells in the lateral magnocellular group of PVN throughout the whole brain area showed triple immunoactivity. In conclusion, this study demonstrates that some of the CNS oxytocinergic cells and gastric motor neurons share their origins from the same cell.

640.2


Blockade of GABACergic tone in the dorsomedial hypothalamus (DMH) increases intestinal motility (Greenwood and DiMarco, Am. J. Physiol. 268:G514-G521, 1995). Hypothesis: Microinjection of bicuculline methylchioride (BMI) into the DMH decreases gastric motor function. Methods: BMI (25 pmol) was microinjected into the DMH of achloroanesthetized rats. Intragastric pressure (iP, cmH2O), pyloric mural motility index (PMMI), and blood pressure (BP, mmHg) and heart rate (HR, bpm) were measured before and after microinjection with student's t-test. Results: BMI DHM NEAR DMH OUTSIDE DMH

<table>
<thead>
<tr>
<th>iP (mmHg)</th>
<th>BMI (N=6)</th>
<th>BMI (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak iP</td>
<td>0.0±2.01</td>
<td>0.4±15.1</td>
</tr>
<tr>
<td>PMMI</td>
<td>0.0±2.0</td>
<td>0.3±1.1</td>
</tr>
<tr>
<td>HR</td>
<td>152</td>
<td>152</td>
</tr>
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</table>

PM < 0.05 vs saline control. Atropine (0.5mg/kg i.v.) abolished the effect of BMI microinjection into DMH on DHM. (0.4±2.3) but not on BP (19±2) and HH (38±1). Conclusions: BMI microinjection into DMH, but not into the surrounding areas, significantly increases iP through a vagally-mediated pathway. Thus, modulation of resident GABAergic tone in this hypothalamic nucleus may contribute to stress-evoked effects on gastric motor function. Supported by PHS grant DK 42714 and the A.P. Sloan Foundation.

640.3

EVIDENCE OF THE INVOLVEMENT OF GASTRIC VAGAL AFFERENTS ESOPHAGEAL COLLATERAL IN A REFLEX ACTION: SHORT VAGO-VAGAL REFLEX. J.Y. Wen*, Y.H. Wang, Y. Tachik* and V.L. Go, Dep. of Medicine, Brain Res Inst and CURE/Gastroenterol Bldg UCLA, Los Angeles, CA 90024-1282.

Esophageal distension (ED) has been shown to induce gastric relaxation, and vagal cooling does not completely abolish this effect (Dig Dis Sci 34:873-881, 1989). We have reported that gastric vagal afferents may emit esophageal collateral nerve branches to the stomach (J Auton Nerv Syst 1995, in press). Aim: using an isolated gastrointestinal in vitro preparation, to determine if short-term lower esophageal distension (ED) can alter intragastric pressure (iP).

Overnight fasted, urethane anesthetized rats were used. Three ligatures were made at cervical esophagus, pylorus and esophago-gastric junction but the gastric nerve was kept intact. A double-lumen catheter was inserted into the stomach for inflating, draining saline and measuring iP. The basal iP was kept at 4 ± 3 mm H2O. A catheter with latex balloon was placed at lower thoracic esophagus and distended with a bolus of saline for 5 sec. The iP (mean ± SE; mm H2O) was decreased 0.74 ± 0.09 (N=8) for 0.5 sec after) all ED: 0.5± 0.04 (N=10), 0.50 ± 0.08 (N=6) and 0.42 ± 0.08 (N=7) for 0.4, 0.3 and 0.2 sec of ED, respectively (p<0.05 ANOVA). Bilateral vagotomy reduced the effect of 0.5 ml ED from 0.74 ± 0.09 to 0.36 ± 0.01 (N=4), whereas a further bilateral splanchicotomy reversed, instead of abolishing, the effect of 0.5 ml ED from 0.36 ± 0.01 to 0.45 ± 0.03 (N=3) (p<0.05, ANOVA) indicating the splanchic nerve provided a tonic excitatory influence to iP. These results indicate that a short-term ED can induce gastric relaxation. About 50% of this effect is CNS-dependent, probably via long vago-vagal reflex, whereas the other 50% can be maintained without CNS and ENS involvement, probably via possal vagal gastric collateral reflex arc, short vago-vagal reflex. (Supported by NIH Grants NS 26433 & DK30110)

640.4

PYO CONTAINING NEURONS AND TERMINALS IN THE NUCLEUS RELATED TO THE AUTONOMIC REGULATION IN RATS. H. Yang*, L. Hwang, H. Wang, and Y. Takedo, CA Dept. of Medicine, UCLA, Los Angeles, CA 90073, U.S.A.

We previously reported that microinjection of PY into mediatory nucleus related to the vagal regulation stimulated gastric acid secretion (LAP 268, 1995). In this study, using a specific polyclonal antibody against PY (CURE antibody Core, #9152), immunohistochemistry was performed to examine the location of PY-positive neurons and terminals in the nuclei related to the autonomic regulation in excitatory treated rats. In the medulla, high density of PY-immunoreactive nerve terminals and fibers were observed in the dorsal vagus complex and raphe pallidus throughout their caudal to rostral extension, while moderately dense networks were found in the area postrema, raphe obscurus (Rob), nucleus ambiguous and the nearby reticular formation. A group of large cells were located in the ventrolateral medulla area throughout the whole caudal to rostral extension as the Rob. In the cells in the caudal part of this cell column were a dense group but gradually became scarce as it extended rostrally. A few PY-positive cells were observed in the rostral part of the nucleus solitary tract. Another large group of neurons was located in the caudal pons at the part between the facial nervere and the Rob. These cells were small cells scattered within 1 mm in both sides along the rostrum. A moderate terminal network and a few cells were located in the locus coeruleus in the pons. PY-containing fibers formed a loose distributed network in the peri-aqueductal gray in the midbrain. In the hypothalamus, dense PY-positive terminals were located in the paraventricular nucleus and arcuate nucleus while moderate dense fibers were observed in the lateral hypothalamic nucleus, supraoptic nucleus and median eminence. A few cells were observed in the arcuate nucleus and the area dorsolateralis to the ventromedial hypothalamic nucleus. Small PY-positive cells and fibers also scattered in the amygdaloid nucleus throughout its caudal-rostral extension. These results indicate that centrally originated endogenous PY may play an important role in the autonomic regulation.
BOMBESIN-LIKE IMMUNOREACTIVE (LI) NERVE TERMINALS IN THE DORSAL VAGAL COMPLEX (DVC) IN RAT, AN ELECTRON MICROSCOPIC ANALYSIS. Richard B Lynes*, Lesley S. Rechcigl1, Richard R. Miselis2. 1Dept. of Medicine, Thomas Jefferson Univ., Philadelphia, PA 19107; 2Dept. of Animal Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Bombesin inhibits gastric function and induces satiety when micro-injected into the DVC. At the light level bombesin is in apparent nerve terminals located in the bowel submucosa of the dorsal nucleus of the vagus (DMV) and the nuclei of the solitary tract (NTS). Electron microscopy was used to determine if synaptic contacts were present. We used a preembedding immunoperoxidase technique with bombesin antiserum (N.E. Potchen, M.S. Lynes, R.R. Lynes, unpublished). Tissues were processed and examined using a Zeiss 10 TEM. In the DMV, bombesin-LI nerve terminals typically contacted small to medium sized dendrites (0.5-1.7μm). Occasional bombesin-LI terminals were associated with a full range of postsynaptic structures including large (proximally dendritic, small dendrites, perikarya and rarely axon terminals. Of 65 axo-dendritic contacts studied in the DMV there were 38 asymmetric and 15 symmetric synapses, and 12 nonsynaptic close approaches. Similar to other neuropeptides, the bombesin-LI reaction product was predominantly over dense core vesicles located in regions distant from the synaptic junction. Bombesin-LI nerve terminals in the NTS have a similar profile of contacts. Terminal contacts in the DMV are of the axo-dendritic type. This study provides further evidence that bombesin is a neurotransmitter/neuromodulator in the DVC, potentially effecting vagal motoneurons. Support: DK02949(RL), GM27739(RM)

640.7


The nucleus tractus solitarius (NTS) is one of the principal sites by which the vagus nerve transmits afferent information from the periphery to the central nervous system and vice versa. It has been shown that bombesin administered directly to the NTS enhances sympathetic nerve activity evoked by stimulation of the vagus nerve (Lundborg et al., 1989). The purpose of this study was to examine the effect of bombesin on the vagal afferent transfer to the NTS. Intracisternal injections of bombesin were ulcerogenic in the stomach (Tache, 1982) and bombesin microinjected into the dorsal vagal complex inhibited gastric motility in rats (Hoyman-Monnikes et al., 1990). These studies suggest that bombesin may play important roles in the control of visceral functions. The present study was carried out to test the effects of intracisternally administered bombesin (0.01, 0.01, and 0.1 μg) on the effect of afferent nerve activity recorded in the NTS. As a result, we found different degrees of bombesin-like peptide in the NTS and we speculate that bombesin may be a neurotransmitter/neuromodulator in the NTS, potentially effecting vagal motoneurons. Support: DK02949(RL), GM27739(RM)

640.8


Peptide YY (PYY) is a 36 amino acid peptide synthesized in the intestinal L cells. It has been proposed that PYY crosses the blood-brain barrier and modulates the central nervous system (CNS) via vagal pathways. We have demonstrated that central injections of PYY inhibit gastric emptying and inhibit gastric acid secretion (Ragosta et al., 1986). In this study, we examined the effects of intravenous, intracerebroventricular and intracisternal injections of PYY on gastric emptying and acid secretion. A 0.25 mg/kg dose of PYY was given intracisternally and a 2.5 mg/kg dose was given by intravenous or intracerebroventricular routes. The results showed that central injection of PYY inhibited gastric emptying and acid secretion. In conclusion, PYY decreases gastric emptying and acid secretion by acting on vagal afferents. Support: DK 42022, NT 11911, GM 11405, MH 33331.

640.9


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

640.10


Intragastric laminar endings (IGLs) are prominent structures of vagal afferent origin with uncertain anatomic distribution. IGLs are particularly in the esophagus and stomach (Berthoud & Neuhuber, 1984). Combining anterograde tracing, immunocytochemistry, confocal laser scanning and electron microscopy, we gained more insight into structural details of IGLs indicative of a possible function. Rat nodule gastrica were injected with WGA-HRP or DII and labeled was seen in the esophagus. Tissues were processed for electron microscopy (WGA-HRP) and immunocytochemistry for calbindin (Cal). Esophag mucosa from untreated rats or animals subjected to unlabeled nerve stimulation were processed for immunocytochemistry for calbindin/calbindin alone, or in combination with S-100 or vimentin. Results indicate that: 1) IGLs are the only significant vagal afferent terminal structures in the striated muscle coat of the esophagus; 2) they are located mainly on the surface of myenteric ganglia immediately beneath the basali lamina. They contain numerous mitochondria, small clear vesicles and a fine filamentous "receptor matrix"; 3) IGLs interdigitate with fine glial processes co-staining for both S-100 and vimentin which probably provide a three-dimensional framework connected to the perginaglonal extracellular matrix; 4) IGLs co-stain for both calbindin and calbindin, two markers which are typically found in rapidly adapting mechanoreceptors (Duc et al. 1993); 5) they form synaptic contacts with enteric neurons. Taken together, these data may indicate a mechanosensor function of IGLs which may be physiologically influence enteric neurons as previously suggested (Neuhuber 1987). (Supported by SNF 3.555-86 and DFG/SFB 553/86)
641.1  

Metabolic and feeding functions have been correlated with the hepatic branch of the vagus and attributed to the liver, in part for lack of information about the branch's non- hepatic projection fields. Although hepatic motor projections to the GI tract have been partially mapped (AP, 220, R200-7, 1991), neither the nerve's afferent projections to the brain or its functional role has been described. To survey these afferents, male SD rats (n = 11) were given partial subdiaphragmatic vagotomies sparing only the hepatic branch and then injected i.p. with Fluoro-gold (1mg). Each rat was perfused and the left vagal segment was excised for histological analysis.

641.2  
**ACTIONS OF CHOLECYSTOKININ IN GANGLIA OF THE GUINEA PIG SPHINCTER OF ODDi. A. P. Gokcek* and G. M. Mawe**.  
*Dept. of Anatomy & Neurological Sci., Univ. of Vermont, Burlington, VT, USA 05401; *Rogomolotz Inst. of Physiology, Kiev, Ukraine, 252024.

Following meals, cholecystokinin (CCK) acts in the sphincter of Oddi (SO), as well as in the gallbladder, to promote the flow of bile into the lumen of the intestine. Motility studies indicate that CCK's action in the SO is neural; however, the neurotransmitters that are involved have not been determined. Three types of neurons based on electrical properties (Wells and Mawe; Am. J. Physiol. 265: G258-H269, 1993), and they contain distinct groups of neurons that express excitatory or inhibitory neurotransactive compounds (Wells et al., J. Comp. Neurol. 352:106-116, 1993). To assess CCK's actions in SO ganglia, neurons were made from 85 neurons in intact SO preparations, and CCK was applied by pressure ejection (0.1 mM, 20 PSI, 0.01 - 3.0 sec) or superfusion (1 - 100 mM). TTX was used to direct excitatory effect on tonic and phasic cells in SO ganglia. In tonic cells (n = 23) CCK typically caused a prolonged depolarization that was accompanied by the generation of action potentials at high frequency during most of the depolarization. In phasic cells (n = 53/55), CCK caused a brief, slight depolarization that was followed by increased excitability. In AH cells, CCK sometimes caused a slight depolarization(n = 3/7), and it caused an increase the amplitude and duration of the prolonged afterhyperpolarization. In summary, CCK has a direct, excitatory effect on the majority of SO neurons. Further studies will be required to determine whether CCK acts on SO ganglia action results in an increased output from inhibitory neurons to relax the SO, and/or increased output from excitatory neurons to pump bile through the SO. Supported by DK 45410 & NS26995.

641.3  
**SYNAPTIC TRANSMISSION IN RABBIT PANCREATIC GANGLIA. T.A. Lent*, Dept of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216.

Pancreatic ganglia are innervated by vagal, sympathetic, myenteric, and primary afferent nerves and provide the postganglionic parasympathetic innervation to pancreatic endocrine and exocrine cells as well as to the intrapancreatic duct. Precious little is known of the synaptic transmitters and resulting potentials which regulate the firing of pancreatic nerves and thus parasympathetic output to the secretory cells. To study these neurotransmitter portions of rabbit pancreas, we used intracellular recording and fluorescent dye injection techniques. Single stimuli of attached nerve fibers evoked nictitating fast synaptic responses and action potentials with repetitive stimulation also revealed slow cholinergic and non-cholinergic EPSPs. Low frequency (5 Hz) nerve stimulation resulted in variable synaptic responses with frequent failures. Atropine (2mM) increased the mean synaptic response while reducing failures. Exogenous muscarine (10mM) decreased the mean synaptic response and social failure. D-100 and at 60 higher stimulating frequencies (1-10 Hz) facilitation of synaptic transmission occurred resulting in frequent action potentials with few failures even in the absence of any slow EPSPs. Application of noradrenaline (10-8M) abolished a firing pattern. Single stimuli of attached nerve fibers evoked nictitating fast synaptic responses and action potentials with repetitive stimulation. We have used a variety of synaptic mechanisms mediated by multiple neurotransmitters to determine the output of pancreatic nerves to the endocrine and exocrine pancreas. Supported by NIH.

641.4  

Prior studies have suggested that N-methyl-D-aspartate receptors (NMDARs) are present in the pancreas. The present study was carried out to (i) locate NMDARs in this organ, (ii) determine if glutaminergic neurons are present in pancreatic ganglia; and (iii) determine whether visceral NMDARs can mediate long term potentiation (LTP). Glutamate (Glu) was detected immunocytochemically in pancreatic neurons in the guinea pig and rat. All of the neurons that were Glu-immunoreactive (Ir) were co-stained with antibodies to Ca²⁺-AT. Glu-Ir nerve fibers were found traveling along blood vessels and in the vicinity of acini. The majority of neurons in pancreatic ganglia expressed NMDARs. In addition, NMDARs were expressed by insulin-immunoreactive islet cells. NMDARs detected pancreatic neurons and induced them to spike repetitively; this effect was blocked by the selective NMDA receptor antagonist, D-AP5 (50mM) and Mg²⁺ (1mM). Pancreatic neurons that received an NMDA were surrounded by Glu-Ir terminals. High frequency stimulation with trains of pulses (ten at 100Hz, 100msec), delivered to intrapancreatic ganglionic connectives, potentiated the amplitude of EPSPs to 138% of control. This potentiation lasted for 50 min and was blocked by the noncompetitive NMDA receptor antagonist, MK801. These observations are consistent with the idea that Glu and NMDARs participate in fast neurotransmission in pancreatic ganglia. The data are also consistent with the possibility that the synaptic release of Glu can stimulate neurons to induce LTP. Supported by grants NS01582 and the American Diabetes Association.

641.5  

The purpose of this study is to assess the role of capsaicinsensitive primary afferent fibers in the inhibition of rat jejunal alanine absorption by vasoactive intestinal peptide (VIP). Continuous intravenous infusion of VIP (112 ng/kg/min) reduced alanine absorption by 66% in control rats and 24% in rats neonatally treated with bilateral capsazepine. VIP decreased alanine uptake by jejunal strips in a dose dependent manner. In the presence of 20 nM VIP, alanine uptake by full thickness jejunal strips was reduced by 53% in control rats and by 19% in rats neonatally treated with capsazepine (p<0.05). On the other hand, the effect of VIP on alanine uptake by mucosal scrapings was similar in both groups of rats. In rats whose jejuna were pretreated with benzalkonium chloride (BAC), VIP's inhibitory effect on alanine uptake was markedly reduced. Kinetic analysis of jejunal alanine uptake in the presence and absence of VIP revealed that it reduced Na dependent alanine transport by decreasing the affinity of its transporter. We conclude that VIP's inhibition of alanine uptake is neurally mediated and involves a change in the affinity for alanine uptake. (Supported by grants from Univ. Res. Board and D.T.S. Fund).

641.6  
**LOCALIZATION OF CALCIUM RECEPTOR EXPRESSION IN CELLS OF THE RAT MYENTERIC PLEXUS. K.V. Rogers, M.C. Dunn, M. Chin, G.C. Jaegers, D. Snyder, E.M. Brangan, 1) NPS Pharmaceuticals, Inc., Salt Lake City, UT 84108, and 2) Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

We have previously mapped the expression pattern of calcium receptor (CaR) mRNA in cells in specific regions of the CNS and have postulated that the ability to sense changes in extracellular calcium may play an important functional role in a subset of CNS neurons. Systemic administration of hypocalcemia is associated with disorders of intestinal motility and neurons in the myenteric plexus play a critical role in the regulation of GI peristalsis. We have localized expression of CaR mRNA, using in situ hybridization, and CaR protein, using immunocytochemistry, in cells of the myenteric plexus of the rat GI tract. Myenteric plexus was obtained from five rats, and Colin proximal, colon, and distal colon were analyzed for rat CaR mRNA. We found that CaR mRNA was detected in all of myenteric regions. Sections of these same regions were hybridized with a 35S-labeled riboprobe complementary to the rat CaR (antisense probe) or a control (sense strand) riboprobe. A subset of cells in the myenteric region of the rat GI tract stained with the antisense CaR riboprobe, while the control did not. These observations suggest that CaR expressing cells in the myenteric plexus may play a role in the regulation of GI peristalsis, in the regulation of the activity of these cells throughout the length of the GI tract suggests that the CaR may be involved in the coordination of motility between different regions of the intestine.
641.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE AND N-METHYL-D-ASPARTATE RECEPTORS IN THE ENTERIC NERVOUS SYSTEM: IMMUNOREACTIVITY IS PRESENT IN BOTH THE MYENTERIC AND SUBMUCOUS LAYERS, NOS/VIP MOTOR CELLS AND CIRCULAR BODIES IN THE COLON.

Prior studies have suggested that N-methyl-D-aspartate receptors (NMDARs) are present in the enteric nervous system (ENS). The present study was carried out to (i) locate NMDARs in the gut, and (ii) determine if glutamate-immunoreactive (Glur) neurons are present in the enteric nervous system (ENS). Glutamate (Glu) immunoreactivity was detected in neurons in the submucosal and myenteric plexuses in all regions of the rat and guinea pig bowel. Glu immunoreactivity was also observed in paravascular nerves and nerve fibers in the enteric plexuses, longitudinal muscle and mucosa. In the guinea pig submucosal plexus, ~ 27% of neurons were Glur. All of the submucosal neurons that were Glur were co-stained with antibodies to choline acetyltransferase (ChAT), substance P (SP), and calretinin, which was present in ~80% of the Glur neurons. The majority of neurons in the ENS expressed NMDARs and, in a subset, there was coincident expression of NMDARs with Glu. These observations are consistent with the idea that enteric Glu and NMDARs play a role in the function of the ENS.

The data are also consistent with the possibility that Glu may be a marker of submucosal intrinsic primary afferent neurons. Supported by NIH grants NS01502, NS12489 and the American Diabetes Association.

641.9 CHEMICAL CODING OF NEURONS IN THE SMALL AND LARGE INTESTINE OF THE MOUSE. H.M. Young*, Q. Sang and J.B. Furness. Department of Anatomy and Cell Biology, University of Western Australia, Nedlands, WA 6009, Australia.

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


The effects of lumbar sympathetic nerves (lumbar colonic nerves, LCN) on non-cholinergic contractile responses evoked by electrical stimulation of the pelvic nerve (PN) were studied in situ by isotonic force measurements of circular and longitudinal muscle slices of cat colon muscle preparations. The proportion of a free-moving smooth muscle sheet (2.5 x 4.5 cm) cut mid-digital colon, the slices were placed in a LCN and isolated from the preparation. The smooth muscle preparation was pinned flat to the bottom of an organ bath (85 ml). Two muscle strips (2 mm wide and 3.4 mm long) were cut parallel to the longitudinal and circular muscle layer, respectively. The ends of each muscle strip remained attached to the smooth muscle sheet. Force transducers were attached to the middle of the muscle strip for bridge-lead recordings of mechanical activity from the longitudinal and circular muscle layer. Bipolar platinum electrodes were placed on the LCN and the organ bath. Electrical stimulation (2-5 Hz 30 s) of the PN caused contraction. The strips were then stimulated with nitric oxide (NO)-dependent non-cholinergic contractions. The inhibition of NO stimulation was abolished by guanethidine (50 μM). In summary, the data show that nitric oxide-dependent non-cholinergic responses of longitudinal and circular muscle are regulated by adrenergic fibers in LCN.


PRV tracing was used to identify sites in the CNS involved in the neural control of colonic function. PRV (7x10⁶ pfu/ml; 1 ml at three sites, 1.5", from anus) was injected unilaterally into the wall of the distal colon and the distribution of PRV-infected (PRV-1) neurons was examined (72,84,96 hr, as each time). In the L1-S1 spinal cord (C), no presynaptic or synaptic varicosities were found in the afferent pathways of the NOS/NPY neurons. In the L1-S1 spinal cord, the majority of PRV-1 cells were present in S1 C. In the (i) lateral paracentral (spinal) nucleus (72-96 hr), 2 dorsal commissures (DCM; 72-96 hr) and 3 dorsal horn (DH; 84-96 hr). The number of PRV-1 cells in the spin was greater than that in the L1 by a minimum of 5.1% at 72 hr. In the T1-L3 DRG, PRV-1 cells were present in: 1) the intermedio lateral cell column (72-96 hr), 2) DCM (72-96 hr) and 3) DH (84-96 hr). PRV-1 DRG cells were present in the L1, L2, L6 and S1 DRG, however, most PRV-1 cells were found in the L5-S1 DRG. In the S1 DRG, cells were found in S1 DRG. Cells were found in S1 DRG. Cells were also found in NADPH-diaphorase activity (NPY and superficial neurons from the spinal cord). NPY-positive (β) colonic ganglionic neurons (PNGN) make up a much larger group (25%-40%) of the neurons in the parasympathetic PGN compared to NPY-positive parasympathetic PGN (5%-10%). NDN cells were present along the outer, occasional submucosal ganglia and internodal neurons, and internodal neurons of the submucosal plexus. These data suggest that the 5-finger region of the Prv gene contains sufficient information to permit cell-specific tachykinin expression in the PNS.


The aim of this study was to test the hypothesis that basal release of nitric oxide (NO) modulates neurally-evoked ion secretion in guinea pig distal colon. Submucosal/mucosal preparations were mounted in Ussing chambers. Changes in short-circuit voltage and/or net movement of chloride ions were used as an index of active, electrico-osmotic ion transport. Electrical field stimulation (EFS, 10Hz, 15 V, 0.5 msec for 90 sec) evoked a biphasic increase in Isc. Pretreatment with the NO chelator, hemoglobin (Hb; 15 mg/10 ml) significantly enhanced both peaks (pk1: 109.2 ± 2.8, pk2: 118.3 ± 4.8 %, n = 7-10, p<0.05) compared to vehicle (pk1: 93.1 ± 3.9 and pk2: 92.0 ± 3.3 %, n = 7-10, p<0.05). Secretion induced by carbachol (0.1 μM), substance P (SP; 1 μM) and vasoactive intestinal polypeptide (VIP; 0.1 μM) was examined in the absence and presence of Hb. Sequestration of NO significantly enhanced secretion induced by VIP (veh: 41 ± 3, Hb: 72 ± 13 %, n = 7, p<0.05) but had no effect on the carbachol and SP response. The results suggest that tonic NO release modulates neurally-mediated increases in ion secretion in part by suppressing submucosal neural activity and postynaptic VIP receptor activation.

Supported by a NSRAF Research Grant.
64.2 A NON-INVASIVE METHOD FOR QUANTIFYING PAIN-RELATED ACTIVITY IN THE HUMAN PRIMARY SOMATOSENSORY CORTEX

E. Schumann, Dept. Psychology, Class., 7-163, Pomona N.T., 12369-5625.

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

64.2.3 COINCIDENT OR DERMATOMAL THERMALLY INDUCED PAIN AFFECTS VIBROTACTILE SENSITIVITY. L. Maxfield and S.J. Bolanowski, Institute for Sensory Research, Syracuse University, Syracuse, NY 13244-2340.

We have previously shown that heat-induced pain co-localized with vibratory stimuli can substantially increase detection thresholds (mean increase = 7.3 dB) in three of the four tactile channels (P. NIP, PINP1) (Apaner, et al. Somassone. Monon., II: 1994). This study was designed to extend these findings by measuring the latency to detection in the fourth psychophysical channel (NP1), to determine site specificity of this effect, and to assess whether cold-induced pain acts similarly. Human detection thresholds (DATP) were measured on the right most prominent of five normal observers across 15 sessions in which pain site and thermal type was varied. First, thresholds were significantly elevated under heat-induced, co-localized pain with vibrations activating NP2 (P<0.05). Second, heat-induced pain measurably increased thresholds when present within the dermatome at sites coincident to the vibratory stimulus, though this effect was somewhat channel-specific: substantial increases were found for the NP1 and NP11 channels (2.4 dB and 2.9 dB, respectively) but not for NP channel. Third, cold-induced pain elevated thresholds substantially, but to a lesser degree than heat-induced pain (mean increase = 3.6 dB). Pain was never found to induce changes in vibrotactile sensitivity when the painful stimuli were presented outside the dermatome, either ipsilateral or contralateral. Our current results suggest that, because dermal pain affects vibrotactile sensitivity, the locus of the pain-touch effect may be subcortical. Further, the effects across heat-induced and cold-induced pain suggest that the underlying fiber populations act differently in these pain-touch interactions.

64.2.4 INNERSVATION TERRITORY OF SYMPATHETIC EFFECTRENTS IN HUMAN SKIN. M. Schmaeh1, B. Schmaeh2, C. Forster1, M. Rinquamp2, H.O. Handwerker1 and R.E. Folds, Institute of Clinical Neurophysiology, University of Upsala, Sweden and *Department of Physiology and Experimental Pathophysiology, University of Erlangen-Nürnberg.

Microneurographic techniques were employed to record from C-fibers in the peroneal nerve of healthy volunteers. The units were identified by their constant latency response to electrical stimulation of the skin (in the skin). Conduction velocities were lower (7.5 ± 0.5 mm/s) than those of afferent units. Most of them were located in the border area of the leg and distal part of the foot dorsal. Sensitopgraphic activity in these units was absent or that low that inherent territories could be mapped by means of trains of transcutaneous electrical stimuli. Two units had 2 and 4 separate territories, resp. Mean innervated area was found to cover 133 mm2 (24-255 mm2). Innervation territories of sympathetic units were about of the same size throughout different regions of the leg and foot including the toes. The two units could be tentatively classified as vasconstricotor and a vasmotor unit respectively. The conclusion we draw is that microneurographic techniques can be used to delineate cutaneous innervation territories of sympathetic afferents. A rough estimation of innervation density for sympathetic units was derived.

64.2.5 SEX DIFFERENCES IN PAIN PERCEPTION AND PAIN ESTIMATION OF CEREBRAL ACTIVATION PATTERNS DURING NOCICEPTIVE THERMAL STIMULATION. P. Paulson1, M. Shinomiya, T. Morrow and K. Casey, Dept. of Neurology, Physiology, and Division of Nuclear Medicine, University of Michigan and Neurology Research Laboratories, VAMC, Ann Arbor, MI 48105.

Sixteen healthy subjects, matched for age and handedness received repetitive innocuous, (40°C) and nocuous (50°C) 5 sec thermal stimuli to the left forearm. Each subject rated the intensity of each stimulation series according to a magnitude estimation procedure in which the 0 = no heat sensation, 7 = barely painful and 10 = terribly intolerable. Sex differences in pain perception were tested for significance using the Student 2-t criterion for each experiment and averaged across subjects of the same sex. Statistical thresholds were applied to each correlation for multiple comparisons (see text for details). Three of the four cortical channels, namely, the primary somatosensory cortex (SI), the precentral gyrus (PRE), and the supplementary motor area (SMA) were selected by standardized stereotaxic coordinates. Subtraction images were created between conditions for each subject and averaged across subjects of the same sex. Statistical thresholds were computed with corrections for multiple comparisons (see text for details). Some of the three subjects showed a trend for enhanced CBF in the primary frontal cortex and lateral hemisphere, ipsilateral thalamus and cingulate cortex compared to the males, these differences were not statistically significant (P<0.05, rectilinear threshold 3.6), perhaps due to the small sample size. These results indicate that males and females perceive pain differently and suggest these differences may be associated with differences in the neural mechanisms underlying pain processing in the primary somatosensory cortex. These data suggest that activity in thermal pathways can contribute to temperature localization. On the finger tips, tactile localization is superior to thermal localization, but on the forehand, thermal localization may be as accurate as tactile. Supported by the Canadian MRC and Quebec FRSQ.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
PAIN MODULATION: ANATOMY AND PHYSIOLOGY—HUMAN STUDIES

643.1 AFFERENT CONDITIONING PRODUCES DIFFERENT EFFECTS ON CLINICAL AND EXPERIMENTAL PAIN IN CHRONIC LOW BACK PAIN PATIENTS. K. Grading and C. N. Y. Hsu-Chen. School of Physical & Occupational Therapy, McGill University, Montreal, Canada. H3G 1V5.

The object of this study was to determine whether chronic clinical pain, acute experimental pain, and the flexion reflex (FR), would be modified in a similar or different manner by noxious transcutaneous electrical nerve stimulation (TENS) in chronic low back pain patients. Thirty young subjects suffering from low back pain longer than six months were studied. They were matched with respect to gender and severity of pain, then randomly assigned to two groups, receiving either TENS or sham TENS to the lumber-sacral region for 30 min. The FR was elicited by a maximally tolerable electrical stimulation applied to the subject's right sole, and recorded electromyographically from the tibialis anterior and sartorius muscle. Subjective pain sensation of the low back pain (clinical pain) and the electrical stimulation (experimental pain) were measured by two separate visual analogue scales. ANOVA and Tukey's tests were used to analyze the data obtained before, during and, up to 60 min after TENS of sham stimulation.

The pre-stimulation control value of subjective low back pain sensation was decreased to 62% in the TENS group (p<0.01), but not in the placebo group. In contrast, there was no significant decrease in both the experimental pain sensation and the FR before, during and after TENS or sham stimulation within each group, and between the two groups. These results show that acute and chronic pain were modified by TENS in a different manner. It is known that the neural pathways mediating the two types of pain are different. Our present findings suggest that there could also be different anti-nociceptive mechanisms for chronic and acute pain in chronic low back pain patients.

643.2 EFFECT OF CAPSAICIN ON HUMAN JAW REFLEX EVOKED BY ELECTRICTOOTH PULP STIMULATION. 1. P. Komposch*, J. Vatima, P. Halimo, H. Kinomura and A. Perkovic. Dept. of Prosthetic Dentistry and Dept. of Physiology, Univ. of Helsinki, Finland.

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


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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBF</td>
<td>Morphine</td>
<td>22.5±9.5</td>
<td>22.6±7.7</td>
<td>19.3±7</td>
</tr>
<tr>
<td>HR</td>
<td>Morphine</td>
<td>156±13</td>
<td>154±13</td>
<td>151±13</td>
</tr>
<tr>
<td>No morphine</td>
<td>161±11</td>
<td>168±19</td>
<td>167±17</td>
<td></td>
</tr>
</tbody>
</table>

(1P <0.05 vs baseline & morphine group.)

Respiration and O2 saturation changed minimally in both groups. Without morphine SBF changes were minimal during PCV placement. SBF changes returned to baseline during recovery in both groups. We conclude: 1) IV morphine prevented a substantial SBF increase from pain, perhaps reflecting an analgesic effect; 2) non-invasive SBF can serve as a measure of pain and analgesia.

643.4 EFFECT OF ACUPUNCTURE ON SYMPTOMATIC KNEE OSTEARTHRITIS. R. H. Wong*, B. M. Berman, L. Luo, P. Leonberg, M. Tishkoff, and M.C. Hobligg. Departments of Family Medicine, Epidemiology and Preventive Medicine, and Medicine, University of Maryland School of Medicine, Baltimore, MD 21201.

Osteoarthritis is a common disease in which there is no specific or curative therapy. The aim of this study was to compare self-reported pain, stiffness and physical function for patients receiving acupuncture plus conventional therapy or conventional therapy alone in patients with osteoarthritis of the knee and to assess the possible adverse effects of acupuncture. Seventeen patients, aged 26-83 years, with moderate to severe osteoarthritis of the knee (fulfilling the American College of Rheumatology (ACR) classification criteria) were enrolled and randomized to either a control (n=9) or acupuncture group (n=8). The control group received standard non-pharmacological treatment. The acupuncture group also received conventional anti-inflammatory and symptomatic treatment. Treatment was twice a week for eight weeks with acupuncture. Outcome assessments of pain and physical function using both the Western Ontario and McMaster Universities (WOMAC) and the Lequesne Osteoarthritis Indexes, and a fifty-foot walk time were performed at baseline and four-week intervals with follow-up at weeks 3, 6 and 8. The WOMAC Osteoarthritis index indicated that the acupuncture group improved significantly at weeks 4 (p<0.05), 8 (p<0.02), and 12 (p<0.05) using Wilcoxon's signed rank test for paired data with two-tailed P values. The Lequesne Index also showed significant improvement at week 8 (p<0.05) for the acupuncture group. There was no significant difference found in the fifty-foot walk times, and no significant adverse effects reported after acupuncture treatment. For the control group, no significant differences were found in the outcome assessments. The results indicate that acupuncture is associated with significant improvement in pain and physical function in patients with osteoarthritis of the knee.

Support provided by NIH R21 AA1-21 RR02137-61.
643.5
GABAB AGONIST, BACLOFEN, REDUCES CENTRAL PAIN WITHOUT IMPAIRING SOMATIC SENSATION. A. Ueda, Y. Kawakami, M.J. Laird. Department of Neurosurgery and Physiology, Tokyo Women's Medical College, Tokyo, Japan. Baclofen, a GABAB agonist, has been shown to produce central pain relief in post-stroke patients. However, the effect of baclofen on the sensory system has not been well studied. In this study, we investigated the discrepancy in effects of baclofen on pain and somatic sensation. Field potentials evoked by sciatic nerve stimulation were recorded in the dorsal horn of the spinal cord in anesthetized rats. Early components (within 2 ms latency) of field potentials evoked by low intensity stimulation were not affected by application of 15 μg baclofen. The early components of field potentials recorded in this experiment may involve single or a few synapses. In contrast, the amplitudes of late components (80 ms latency) evoked by high stimulus intensity decreased to 55% of the control value. Next, using the H-reflex, we demonstrated that 15 μg baclofen had no effect on H wave amplitude. The results suggest that baclofen, in the spinal cord, has little effect on single synaptic responses. This may account for the discrepant effects on pain, somatic and motor control.

643.7
CHANGES IN SKIN AND MUSCLE SENSITIVITY IN DYSMENORRHEIC VS NORMAL WOMEN AS A FUNCTION OF BODY SITE AND MONTHLY CYCLE. M.A. Giamberardino*, K.J. Bedeck*, S. Jezek, P. de Boysson, L. Vercelletto. Inst. of Medical Pathophysiology, G. D'Annunzio Univ., Chieti, Italy and Program in Neuroscience, Florida State Univ., Tallahassee, FL, USA. Changes were investigated in skin and muscle sensitivity of 7 dysmenorrheic vs 7 normal women as a function of body site and inside the metanephrine field of reproductive organs and monthly cycle. Pain thresholds to electrical stimulation were measured for two abdominal areas (inside reproductive field—symmetrical sites of the left and right rectus abdominis and overlying skin, 4 cm lateral to the navel) and for the limb areas (outside reproductive field—deltoid and quadriceps and overlying skin of one side). Measurement was repeated 4 times during a 28-day cycle; i.e., on days 2–6, during menstruation (phase a); 12–16, periovulatory (phase b); 17–22, luteal (phase c); 23–28, perimenstrual (phase d).

Presence of dysmenorrhea: Thresholds in dysmenorrheic women were normal in skin but lower than normal in muscle in every site with a significant difference for the abdomen in all phases and for the deltoid in phases a and b. Body site: In dysmenorrheic and normal women muscle thresholds were lower in the abdomen than in the limbs. Cyclic variations: In dysmenorrheic and normal women the highest threshold value was in the luteal phase (phase c) for both skin and muscle while the lowest occurred periovulatory (phase b) for skin and perimenstrual (phases a or d) for muscle. This monthly trend was significant in muscle in every site for dysmenorrheic women but only in the abdomen for normal women. Hypersensitivity is evident in muscle but not in skin both inside and outside the metanephrine field of reproductive organs for dysmenorrheic women but only inside them for normal women. Sensitivity increases periovulatory in skin and perimenstrually in muscle and decreases in the luteal phase for both tissues regardless of body site and dysmenorrhea status, but a significant monthly rhythm appears only in hypogastric areas. (KSB was supported by funds from NIH grant NS 11898; all other authors were supported by funds from CNR-FATMA grant 94.00671.PF41).

644.1
EFFECTS OF UNILATERAL NUCLEUS TRACTUS SOLITARIUS LESIONS ON CENTRALLY-MEDIATED HYPERALGESIA. E.P. Wiersinga* & J. Roeppe, Dept. of Psychology, Macalaster College, St. Paul, MN 55105.

Endogenous hyperalgesia circuitry can be activated in response to a variety of aversive events. Specifically, illness-causing agents (such as emetics or pyrogens) have been shown to produce internal aversive events that result in the enhancement of pain sensitivity as measured by the tail-flick test (TF). Recent evidence has also shown that s.c. formulated (topical) baclofen activates a centrifugal pathway that results in hyperalgesia measurable using the TF test. While the neural circuitry responsible for these centrally mediated hyperalgesias has only recently come to be investigated, current evidence strongly suggests that illness-induced hyperalgesia originates in the viscera at vagal terminals, implicating that the ascending signal may involve mediation in the hind brain at the nucleus tractus solitarius (NTS). The present studies investigated the possibility that lesions (unilateral, electrolytic) of the NTS would disrupt centrally mediated hyperalgesias. After a recuperative period, pain responsiveness was tested in lesioned and sham-lesion control animals following injection of lipopolysaccharides (LPS; intraperitoneal, IP; pyrogenic) lithium chloride (LiCl; IP, iemetic), or formalin (right hindpaw; irritant). Preliminary results indicate that NTS lesions disrupt hyperalgesias produced by LPS and LiCl, but not formalin. Supported by NBNINDA grant 1R29DA09289-01 to EPW.

644.2
INTERACTIONS BETWEEN LOW THRESHOLD MECHANOCEPTORS AND NOCICEPTORS DURING SECONDARY HYPERALGESIA. F. Cavazzini and J.M.A. Laird. Dept. of Physiology and Pharmacology, University of Alcalá de Henares, 28871 Madrid, Spain.

Hyperalgesia states are characterized by an increase in the pain evoked by noocptor stimulation and by a central alteration in the processing of low-threshold mechanoreceptor input such that their stimulation evokes pain (alldynia). In this study we have examined if stimulation of low-threshold mechanoreceptors from an area of secondary hyperalgesia could evoke noociptor activity expressed as local vasodilatations (axon reflexes). Experiments were conducted on normal human volunteers. Cutaneous blood flow and temperature in two different points were measured with a Laser Doppler instrument. Hyperalgesia was induced by the application of mustard oil (25%) or by intradermal injections of capsaicin (25-50 μg) in the solar skin of one forearm. Stimulation of low-threshold mechanoreceptors was achieved by brushing or stroking the skin. Pain ratings (VAS) and simultaneous recordings of blood flow and skin temperature were obtained.

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

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

The activity of modulatory neurons was recorded in rats during application of noxious heat to the tail or noxious pinch to a paw. Thionental anesthesia kept the animals free from signs of pain or discomfort while stable responses to noxious stimulation occurred. Of cells were characterized, since off-cells increased and stop their firing just before tail-flick or immediately after noxious pinch. Thereafter FOR (100 µl, 15%) was subcutaneously injected in one of the hindpaws and the responses of the on- and off-cells were studied for 65 min.

FOR produced both early and late excitatory responses on the firing frequency (359.64 and 242.54%) respectively in all the on-cells tested, but only inhibition of activity (61.12%) during the early phase in the off-cells. During the late phase the off-cells showed only a reduction of 16.67% in their firing frequency. This suggests the existence of a new possible balance of activity between the two classes of neurons, probably induced by the nociceptive stimulus in which the suppressive effect of off-cells during the late phase probably is not sufficient to cause inhibition of on-cells. Also, the interphase period, behaviorally defined as a zone of no pain, can be the result of the continuously firing off-cells during this interval in the off-cells.

(Supported by grant SI-2672 of CONICIT.)

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


Evaluation of data from clinical studies show that chronic muscle pain is not associated with tonic hyperactivity, in contrast to nociception theory. Instead, resting EMG activity appears normal. During function, agonist activity is less than normal, while antagonist activity tends to rise slightly. Experimental pain causes similar changes in muscle activity in man and animals. The specific aim of the present study was to evaluate how nociceptive muscle inputs act on the brainstem orofacial motor circuitry during masticatory movements. Rabbits were anesthetized with halothane, decerebrated at the precentral level and paralyzed. Fictive mastication was evoked by repetitive stimulation of the cetico-bulbar tract and monitored in the trigeminal motoneuron subnucleus. Muscle pain was induced by injections (160 µg) of hypertonic saline (5%) into the deep portion of the masseter muscle. Changes in the masticatory motor pattern and the firing properties of interneurons located in the oral subnucleus of the oral trigeminal tract, were simultaneously analyzed prior to, during and after fictive mastication induced in the presence or absence of the tonic inputs. Our results show that muscle nociception reduces the masticatory activity and increases the antagonist-related EMG activities. An increased firing frequency was the main finding. Our results suggest that muscle nociceptive inputs adapt the movement pattern through actions on the rhythm and burst generating components of the brainstem central pattern generator for mastication.

Supported by a Group Grant from the Canadian MRC, the Swedish MRC and Glo-Swarc-France.


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

Male Sprague-Dawley rats were chronically implanted with a guide cannula aimed at the RVM and injected with low doses of kainic acid (5, 10, and 20 pmol in 200 nl) or saline. Microinjection of kainic acid into the RVM produced a dose dependent inhibition of locomotion in the open field test (i.e. no effect with 5 pmol vs. maximal effect with 20 pmol) even though rats were capable of movement. Moreover, immobility in the open field was consistently associated with antinoception on the hot plate test.

The immobility and antinoception seen in the present study are similar to the effects produced by activating neurons in the ventrolateral PAG and suggest, along with anatomical and physiological data, that these behaviors are mediated via a ventrolateral PAG to RVM pathway. Presumably, the PAG and RVM are part of an integrated defense circuit coordinating responses to potentially life threatening situations. Funded by a grant from the Washington State University Alcoholism and Drug Abuse Program.

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE (TH-IR) APPositionS ONTO PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE RAT ROSTRAL VENTROMEDIAL MEDULLA X. MENG, B. Budes, R. Skarne, H. EM,

Fields, Dept. of Neurology and the William B. Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

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

On- and off-cells were identified by the change in their discharge rate in relation to nociceptive withdrawal reflexes and known nociceptive inputs. Sections containing labeled cells were visualized with a Texas red fluorochrome conjugated to avidine and were subsequently processed for TH-IR using goat-anti-rabbit IgG as a secondary antibody conjugated to a Bodipy fluorochrome. The distributions of the two fluorochromes were then mapped using a confocal scanning microscope (BDG 600).

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

This work provides evidence that putative pain modulating neurons in RVM receive direct noradrenergic inputs.

Supported by NIH grants NS 31495


Microinjection of GABA receptor ligands in the n. raphe magus (NRM) or n. reticularis gigantocellularis pars parvocellularis (NGCP) modulates nociceptive sensitivity. It is not known whether these effects are mediated by spinally-projecting neurons of the NRM and NGCP. This study examined whether neurons of the NRM and NGCP project to the spinal cord and can contain the specific GABA receptor subunits.

Male S-D rats were perfused with 4% paraformaldehyde 10-14 days after injection of 2% Fluoro-Gold (FG) in the spinal cord. Transverse 25 µm sections were incubated with rabbit anti-somatostatin (SST) antiserum, (1-16) subunit or guinea pig anti-alpha-1 (1-15) subunit antibodies. Other sections were incubated in goat anti-SHT antiserum and rabbit anti-α1 (1-9) subunit antibodies. Primary antisera were visualized using CV-3 or FITC-linked secondary antibodies. Neurons in the NRM and NGCP were immunoreactive (IR) for the α1 subunit, but none of these neurons were IR for SHT. Of the α1-IR neurons, a portion projected to the spinal cord as indicated by the presence of FG. NRM and NGCP neurons were also IR for the α2 subunit. Many, but not all of these neurons were also IR for SHT. A portion of both populations of α1-IR neurons contained FG. The differential distribution of α1 and α2 subunits, which confer different pharmacologic properties to GABA, receptors, suggests that NRM and NGCP neurons that project to the spinal cord may be mediated by different types of the GABA receptor. Supported by DE1423 and DA07255.
644.9

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

Corneal stimulation at noxious intensities produces Fos-like immunoreactivity (Fos-LI) in two spatially distinct transition regions of the spinal trigeminal nucleus (Vc/Vc and Vc/Ct). Fos is the protein product of the immediate early gene c-fos and is a reliable marker for nociceptive-responsive neurons in the medial dorsal horn. Morphine and somatostatin have been implicated as putative mediators of antinociceptive behavior among central trigeminal neurons.

To assess the role of mu opioid and somatostatin receptor activation on Fos-LI, morphine (0.01-10 nmol) or octreotide (long-acting somatostatin analog, 0.1-1 nmol, iv) was given alone or in combination 20 min prior to corneal application of mustard oil to male rats anesthetized with pentoobarbital. Mean arterial pressure (MAP) and heart rate (HR) were monitored in all groups. Neither morphine nor octreotide affected Fos-LI at the pericrural Vc/Ct level, in ventrolateral medulla or at NTS. In contrast, both morphine and octreotide caused a dose-related decrease (P < 0.01) in Fos-LI among lamina I-II neurons at the Vc/Ct transition level.

Combined morphine (0.01 nmol) plus octreotide (1 nmol) caused a greater reduction in Fos-LI in lamina I-II at Vc/Ct than the additive effect of each drug alone. Morphine attenuated the MAP and HR responses to corneal stimulation in a dose-related manner, whereas octreotide had no consistent effect when given alone or in combination with the lowest dose of morphine. The magnitude of Fos-LI among lamina I-II Vc/Ct neurons was well-correlated with the MAP response to corneal stimulation under different doses of morphine (r = 0.63, P < 0.01), but not after octreotide. The results indicate that morphine and somatostatin interact centrally to reduce the expression of c-fos among central nociceptive Vc/Ct neurons, but not Vc/Ct neurons.

The neural substrates that mediate the apparent interaction between morphine and somatostatin and c-fos expression is separate from that which mediates the autonomic responses to corneal stimulation. Supported by NIH grant NS 20137.

645.1

REPETITIVE STIMULATION AND NALOXONE FOLLOWING OPIOID EXPOSURE INDUCE NMDA RECEPTOR-DEPENDENT LONG TERM POTENTIATION (LTP) IN ISOLATED RAT SPINAL CORD. L.D. Feng and 1. J. Kendig*. Dept. of Anesthesia, Stanford Univ. Sch. of Medicine, Stanford, CA 94305

Recent evidence suggests that hyperalgesia induced by prolonged noxious stimulation and by precipitated withdrawal from opioids may have mechanisms in common. In isolated neonatal rat spinal cord, a prolonged (30 min or longer) significant increase in area of a nociceptive- related slow ventral root potential (sVRP) was induced by either tetanic (10 s, 1 min) stimulation to a dorsal root (124% of control ± 22.4, mean ± SD, N=28) or by naloxone 200 nM following morphine 200 nM (142±17.5, N=6) or alfentanil 200 nM (148±29.2, N=11). MK-801 20 nM had no effect on sVRP area alone but given before the tetanus or coadministered with the opioids blocked the increase in sVRP area induced by either tetanic stimulation or opioid. MK-801 20 nM shifted the morphine dose-response curve for sVRP depression significantly to the left.

The results are consistent with a prolonged NMDA receptor-mediated excitatory effect exerted by repetitive stimulation and by μ opioid exposure. The phenomenon may represent the first step in a central sensitization that underlies both tolerance and neuropathic pain.

645.2

PHARMACOLOGICAL CHARACTERIZATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN ISOLATED SPIRAL CORD AND NEUROPATHIC RATS. H. Wen, L.B. Jakeman, and D.W. Bonhaus. Synthex Research, Palo Alto, CA 94304

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

However, spinal cord NMDA receptors could be distinguished from those in brain cortex. Sporadically allosterically increased [3H]TCP binding to NMDA receptors in brain cortex membranes (32±9%) but had no effect on [3H]TCP binding to spinal cord membranes (2±1%). In this regard spinal cord NMDA receptors resembled those in the cerebellum (2±9%).

The findings indicate that while there may be no change in the density of spinal cord NMDA receptors in neuropathic rats, spinal cord NMDA receptors can be distinguished from receptors in the cortex. Thus, it may be possible to develop NMDA receptor antagonists with selectivity for the spinal cord.

645.3


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

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

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

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

645.4

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

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

Two groups of experiments were carried out in order to investigate if NMDA receptors were involved in the well known analgesic response induced by the stimulation of the cholinergic or the opioid systems. In a first group of experiments, CD1 mice were injected with the non-competitive NMDA receptor antagonist MK-801 (0.075, 0.1, 0.125, 0.15 mg/kg, i.p.) and 15 minutes later with vehicle or oxotremorine (0.03 mg/kg, i.p.). After 15, 30 and 60 minutes from the second injection, they were tested in the tail-flick test. The results showed no significant effects on pain sensitivity of mice injected with MK-801. Moreover, MK-801 did not significantly modify the analgesic effects of oxotremorine. On the other hand, the results of a second group of experiments showed that the antinociceptive response induced by the administration of morphine (5, 10 mg/kg, i.p.) was potentiated by MK-801 (0.075, 0.1, 0.125, 0.15 mg/kg, i.p.). The present results support the hypothesis that the glutamatergic system is involved in the antinociceptive effects induced by the stimulation of the opioid system, while they seem to rule out that in our experimental conditions NMDA receptors mediate the analgesic response due to the cholinergic activation.
645.5

NMDA ANTAGONIST, MK-801, BLOCKS THE DEVELOPMENT OF TOLERANCE TO PAIN IN INJURED SR IN MICE. John N. Owen*, Michael W. H breit and Anthony L. Vaccaresi. Department of Psychology, University of New Orleans, LA 70128

Several lines of evidence indicate a critical role of the NMDA receptor in the development of tolerance to morphine-induced analgesia. The present study examined the involvement of the NMDA receptor in the development of tolerance to morphine-induced analgesia. For the induction of tolerance, mice were injected with saline or the NMDA receptor antagonist AP5 (20 mg/kg) daily for 20 days. Mice were then subjected to the morphine test on days 21 and 22, and tolerance was assessed by the effects of saline injection on the conditioned回避 response of rats. The NMDA receptor antagonist AP5 significantly reduced the development of tolerance to morphine-induced analgesia, indicating that the NMDA receptor plays a critical role in the development of tolerance to morphine.

645.6


Long-term potentiation (LTP) of C-fiber-evoked field potentials in spinal dorsal horn may underlie the prolonged central changes of nociception. In this work the roles of N-methyl-D-aspartate (NMDA) and neurokinin (NK1 and NK2) receptors for the induction of LTP were evaluated by superfusion of rat spinal cord with selective agonists and antagonists of NMDA and neurokinin receptors. Superfusion of spinal cord with NMDA receptor antagonist D-1-phenylalkylpiperazine (PPQ) or MK-801 (100 mg/ml) for 30 min before and for 30 min after conditioning tetanic nerve stimulation had little effect on the amplitude of C-fiber-evoked potentials but completely blocked LTP in all rats tested. Superfusion of spinal cord with NMDA (100 mg/ml) or AP5 (100 mg/ml) before and for 30 min after tetanic stimulation blocked the induction of LTP completely in all rats tested. Superfusion with SR 48698 (100 mg/ml) depressed the amplitudes of C-fiber-evoked field potentials by 80% but had no effect at 10 mg/ml. The superfusion of spinal cord with AP5 at 100 mg/ml prevented LTP in all rats tested. The superfusion of spinal cord with the NK1 receptor antagonist RP 67505 at 5 mg/ml inhibited the amplitudes of C-fiber-evoked potentials by 50% and had no effect at 1 mg/ml. NK2 receptor antagonist GR 38032F at 100 mg/ml decreased the amplitudes of C-fiber-evoked field potentials by 80% but had no effect at 10 mg/ml. These results indicate that the NMDA and neurokinin receptors are critically involved in the induction of LTP of C-fiber-evoked field potentials in rat spinal dorsal horn. Supported by the Deutsches Forschungsgemeinschaft.

645.7

CONTRIBUTIONS OF NMDA RECEPTORS TO HYPERALGESIA INDUCED BY INJECTION OF MUSTARD OIL INTO THE ANKLE JOINT OF THE SPINAL INFOSUAL RAT. E. Shiok, C.L. Delaney, Dept. of Pharmacology, and Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242 and Dept of Physiology, Universidad de las Americas, Merida, Venezuela.

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

Rats were anesthetized with pentobarbital (45 mg/kg). Their spinal cords were transected at T11, an electrode was placed on the sciatic nerve, and either an intrathecal catheter was inserted at T8 and guided to L1-L2 or a catheter was implanted in the jugular vein. The following day, hyperalgesia was induced by injection of 10 μl of mustard oil into the ankle joint, which caused a rapid onset, long lasting facilitation of the flexion withdrawal reflex elicited by C-fiber intense (10 mA) electrical stimulation of the sciatic nerve and measured by recording the EMG in the hindlimb muscles. NMDA antagonists, APV (1) and ketamine (iv), were administered 21 minutes following mustard injection or, for control experiments, in the absence of mustard oil injection into the ankle.

APV (0.1-100 μm) and ketamine (0-10 mg/kg) dose-dependently inhibited both the hyperreflexive and baseline responses. The ED50 for APV was approximately 20 μmol for both hyperreflexia and baseline responses, while the ELD50 for ketamine was slightly less for hyperreflexic (2 μmol/kg) than for baseline (5 mg/kg). These results demonstrate that NMDA receptors are involved in both acute and mustard oil-induced hyperreflexive nociception in spinal rats.

645.8

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

Modulation of responses of Lp-Sp spinal neurons to colorectal distension (CDD, 20-80 mmHg) was examined following activation of spinal NMDA receptors in pentobarbital-anesthetized rats. Neurons were recorded extracellularly and drugs (1-10 μm) were administered by pressure injection from a microcapillary electrode as described. Administration of NMDA as well as D-serine, but not saline, in the vicinity of neurons responsive to CDD produced significant increases in the magnitude of neuronal responses to CDD as well as neuronal discharge in the absence of CRD. NMDA also increased the gain of the stimulus intensity-encoding functions of neurons to CDD and lowered thresholds for neuronal responses to CDD. Convergent effects of NMDA on visceral somatosensory responses to CRD showed expansion after administration of NMDA or D-serine. All of the above facilitatory effects were produced within 30-45 min of NMDA or D-serine administration and were blocked by coadministration of D-APV or 7-Ck, respectively. Repeated high-intensity electrical stimulation (100 Hz, 30s, 1 Hz) in convergent cutaneous receptive fields of neurons responsive to CRD produced a facilitation of neuronal responses to subsequent noxious CRD in a D-APV-sensitive manner.

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

645.9

2-AMINO-5-PHOSPHONOVALERIC ACID(AP5) BLOCKS COLORECTAL SENSITIZATION BY TURPENTINE Y. Iida,*, Y. Maehara, T. Usukura, J. M. Kitahara, and J.G. Collins. Anes. Dept., Yale University School of Medicine, New Haven, CT 06510.

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

Under pentobarbital anesthesia (40 mg/kg IP), Sprague-Dawley rats were prepared with a laparotomy from T12 to L1 for intrathecal administration of AP5. Physiological parameters were maintained within normal limits. Animals were divided into two groups, control group with 50 μl of saline(NS) and AP5 group(AP5) with 10 mg AP5 in 50 μl saline. Visceromotor response to CRD was determined during control study. After the baseline study, AP5 or saline was administered, and 5 min later VMR threshold was determined. Then 1 ml of 25% turpentine was injected rectally. VMR thresholds were determined every 5 min thereafter for the remaining 90 min. ANOVA and t-test were used for statistical analysis. Sensitization treatment of the same group resulted in a significant decrease in VMR threshold over the 90-min observation period.

In contrast, animals pretreated with AP5 showed no such reduction in VMR threshold during the 90-min observation period. These results suggest that the bowel induced hypalgesia by turpentine produce sensitization involving spinal sensory mechanisms mediated NMDA receptors. (Supported by: NIH Grant NS-09871)

645.10

ADRENALED MedULLARY TRANSLANTS ATTENUATE SPINAL CORD GOMP PRODUCTION IN RESPONSE TO PERIPHERAL NERVE INJURY. J. B. Sigafoos*, A. Hama, and Uagen. Dept of Anatomy and Cell Biology, Univ. of Illinois at Chicago Medical Center, Chicago, IL 60612.

NMDA receptor activation has been shown to result in a Ca2+ dependent increase in cGMP through the production of nitric oxide. In the spinal cord, this cascade of events has been implicated in the persistence of pathological pain following injury. Previous studies in our laboratory have demonstrated that the transplantation of adreanal medulla into the spinal hemicord of neonatal rats can alleviate chronic pain in animal models. The purpose of this study was to determine whether adrenal medulla transplants act via attenuation of cGMP production in spinal nociceptive regions. Neuruporogenesis was induced by unilateral constriction of the sciatic nerve. Two weeks following nerve injury, rats were transplanted with either adrenal medulla or control striated muscle tissue in the spinal lumbosacral area. One week after transplantation, cGMP levels in the spinal cord segments (L4-L5, the region of sciatic nerve innervation) were dissected and assayed for cGMP using routine radiomunoununoassay. Results demonstrated a marked increase in cGMP levels in animals with peripheral nerve ligation, ipsilateral to the chronic constriction injury. The elevated cGMP levels were also found in nerve ligated animals with control transplants. However, chromaffin cell transplants reduced cGMP levels in injured and intact (non-injured) animals. These results demonstrate that cGMP production can be significantly reduced via adrenal medulla transplants, paralleling the transplantation of the cGMP producing nerve. In summary, the results suggest that adrenal medulla transplants may intervene in the cascade of events initiated via the activation of NMDA receptors in pathological pain. Supported by NS25040.
INTERACTION BETWEEN SPINAL NEOSTIGMINE AND CLONIDINE IN SHEEP: ROLE OF NITRIC OXIDE
C. Tęcza, Z. Xu, J. G. Esztergally, The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27109
Several lines of evidence suggest that α2-adrenergic agonists produce analgesia after spinal injection in part by causing acetylcholine (ACh) release. As such, spinal injection of the cholinesterase inhibitor neostigmine, has been shown to enhance spinal choline-dependent antinoceptive effects of spinally released ACh, a mediator in part by nitric oxide (NO). The current study examined the role of NO in the clonidine-neostigmine interaction in sheep. Following approval from the Animal Care and Use Committee, 7 sheep were prepared with chronic lumbar intrathecal (L.T.) catheters. Dose responses to analgesia (foreign withdrawal to noxious mechanical stimulus) were obtained to L.T. clonidine, L.T. neostigmine, L.T. α2-methyl-prazosin (NMLA), L.T. NMLA plus clonidine, and L.T. NMLA plus neostigmine. Treatments were compared by 2-way ANOVA on the dose responses and by 1-way ANOVA on the calculated ED50 for each treatment, with P<0.05 considered significant. L.T. clonidine produced dose-dependent antinoception, whereas L.T. neostigmine and NMLA were without effect. Neostigmine significantly enhanced clonidine antinoception. NMLA did not affect clonidine-induced antinoception, but blocked the interaction between clonidine and neostigmine. These data agree with previous data in sheep that spinally administered α2-adrenergic agonists cause antinoception, whereas there is no evidence of chronic spinal cholinergic tone, such that spinal neostigmine alone causes antinoception. Similarly, lack of effect of NMLA alone on antinoception suggests no chronic release of NO in the unstimulated state. Supported in part by GM55523.

THE ROLE OF NITRIC OXIDE AND SPINAL TACHYCHORDIA IN THE BOLD RELAXATION RESPONSE
C. Tęcza, Z. Xu, J. G. Esztergally, The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27109
We have investigated the contribution of substance P and nitric oxide (NO) to formalin-evoked spinal cord c-Fos expression. Although formalin-evoked c-Fos expression systems are strongly implicated in the spinal processing of prolonged, but less acute, nociception.

This 6 hours after intraplantar formalin Fos-immunoreactive (Fos-LI) neurons (total expression 175/13 neurons per section) were essentially located both in the superficial (94/69 Fos-LI) and deep (80/66 Fos-LI) laminae of the dorsal horn of IL-1β segments of the spinal cord. Systemic administration of L-NMMA (30 mg/kg, i.p.) produced an increase of Fos expression in the superficial and deep laminae (17/38% increase, p<0.05), in this condition nociception was significantly reduced compared to control. These results clearly demonstrate the contribution of both substance P and NO to inflammatory evoked Fos expression at the spinal level, however they also illustrate that substance P influences nociceptive responses in both the superficial and deep laminae of the dorsal horn, whereas NO appears to preferentially influence deep laminae neurons. Since the peripheral inflammatory process was not influenced these effects are probably due to a central site of action.

ANTINOCESSION AFTER AN INTRATHecal INJECTION OF W-NITRO-L-ARGININE (L-NAMe) IN MICE. A.A. Larson* and K. F. Kito, Department of Veterinary Pathology, University of St. Paul, MN 55411, USA.

The present study was designed to determine the role of nitric oxide (NO) in nociception by assessing the effect of L-NAMe, a NO synthase inhibitor, on nociception. Although L-NAMe has been found to inhibit hyperalgesia, antinociception following 5 min of L-NAMe was not observed at 1 or 6 hours after its injection when tested using the hot plate, tail flick or writhing assays. However, 16 and 24 hr after its injection intrathecally, L-NAMe (10 to 100 nmol) produced a dose-related inhibited of peripheral and central cholinergic analgesia, it did not significantly influence formalin-evoked c-Fos expression. Neither L-NAMe or N-NMMA increased peripheral c-fos expression.


Recently it was suggested that nitric oxide could play an important role in peripheral and spinal antinociception. We have investigated the antinociceptive effects of nitric oxide (NO) on nociception in the rat. The present study was designed to find out whether the oxotremorine-induced antinociception was mediated by nitric oxide. In a first set of experiments it has been observed that intrathecal injection of the inhibitors of the nitric oxide synthetase, Nω-nitro-L-arginine methyl ester (L-NAME) and Nω-nitro-arginine (N-ARG), in doses of 10 and 20 mg/kg, had no effect on the thermal nociceptive threshold of male CD1 mice. When administered before oxotremorine (0.005, 0.01, 0.02, 0.03mg/kg), L-NAME and N-ARG significantly potentiated the dose-dependent analgesic effects of the muscarinic agonist. In a second set of experiments intrathecal administration in rats of Wistar strain of L-NAMe (50, 400 μg/Kg) did not change the nociceptive threshold, but dose-dependently potentiated the effects of oxotremore injected i.p. in a dose 0.02 μg/kg, both in paw pressure and in tail flick tests. The NO donor SIN-1 (10,100μg/Kg), without effects when given alone, antagonized oxotremone-induced analgesia in both tests used. The obtained data indicate that nitric oxide exerts an inhibitory action on cholinergic-induced analgesia and evidenced the pronociceptive effects of NO in the spinal cord.

Society for Neuroscience, Volume 21, 1995
645.17
Capsaicin Evokes the Release of L-CCR from Dorsal Horn Slices in a Nitric Oxide Dependent Manner. Mary G. Garry, PhD. Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas, TX 75235

646.2
DIRECTIONAL SELECTIVITY IN RETINAL GANGLION CELLS IS ABOLISHED BY NEOMYCIN. R. J. Jensen, Southern College of Optometry, Southwestern College, Queensland, Australia.

Findings from several studies suggest that the antibiotic neomycin may block voltage-activated P- or Q-type Ca²⁺ channels. In a recent study (Jensen, J. Neurophysiol., In press) it was found that the Q-type Ca²⁺ channel blocker α-conotoxin MVIC abolished directional selectivity in ON/OFF directional selectivity in cat retinal ganglion cells. Extracellular recordings were made from ON/OFF directionally selective ganglion cells in superfused rabbit retinal strips. At 480-800 μM, neomycin abolished directionality in these cells (n = 7) by bringing about a response to movement in the opposite direction. The effect of neomycin was rapidly reversible upon washout. Some findings were obtained with gentamicin, streptomycin, and tobramycin but only at concentrations greater than 1000 μM. In conclusion, neomycin and other aminoglycoside antibiotics appear to block α-conotoxin MVIC-sensitive Ca²⁺ channels in the retina. (Supported by NIH EY07318)
646.5 PHYSIOLOGICAL PROPERTIES OF AXON BEARING WIDE FIELD AMACRINE CELLS IN THE RABBIT RETINA. W. K. Taylor and H. Wässle. Max-Planck-Institut für Hirnforschung, D-60728 Frankfurt, Germany.

Axon bearing wide field amacrine cells in the rabbit retina have a central sparse axon from which several axon-like processes emerge. The axons are generally straight, randomly oriented and can be several millimeters in length (Vaney et al., 1981). We address two questions important to the physiology of these cells. 1. Do the cells generate action potentials? 2. How large is the receptive field?

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

Action potentials were elicited during depolarization. The receptive field center had a diameter similar to that of the dendritic arbor. There appeared to be an excitatory central region and a larger inhibitory surround. The cells generated transient excitatory responses in response to a light step. The excitatory current decayed with a time constant of about 5 ms.

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

Supported by grants NEI08848 and NEI07738, and KRODS, HB30772. KRO is a Jules and Stein Professor of RBPI Inc.


Spatial correlation in a natural scene is exponential. Therefore, cone signals contributing to a receptive field center are strongly correlated and those contributing to the surround are spatially uncorrelated. Surprisingly, the center signals improves the signal/noise ratio (S/N) compared that of a single cone (Tsukamoto et al., '90), but the effect of subtracting the surround signals is unknown. We compared the S/N improvement (I) for the center only and for center-surround. The incorporation of the surround in the computation is known to depend upon cell densities and receptive field parameters, plus scenes of different spatial frequency composition (characterized by the space constant, λ). When high frequencies are abundant (λ<10 mm), S/N is slightly greater for the center-surround than for the center only and correlations between neighboring cells are reduced. The effects of the surround on S/N are nearly constant with eccentricity, due to the relative expansion of the center. S/N is always considerably higher for peripheral than for central cells. Overall design improves encoding by central beta cells of high spatial frequencies (preserved by smooth pursuit eye movements) and encoding by peripheral beta cells of lower spatial frequencies (arising from blur due to smooth pursuit) Supported by EY00828.


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

Supported by grants EY00658 and EY027318, and KRODS, HB30772. KRO is a Jules and Stein Professor of RBPI Inc.

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

The potentials measured by the flicker ERG originate at multiple sites in the retina. To exploit the ERG to understand the flow of information through the retina, it is necessary to develop techniques that allow one to distinguish activity at individual sites or in particular pathways. To this end, we have recorded flicker ERG responses to stimuli whose spectral properties isolate individual classes of cone photoreceptors. Stimuli were presented on a computer controlled colorimeter. The color of the stimulus field was modulated temporally around a fixed mean level of illumination. Control software allowed us to specify independently the modulation contrast seen by human long, middle, and short wavelength sensitive cones. ERG responses were recorded both to cone isolating stimuli and to stimuli that modulated multiple cone classes in various combinations. We began by measuring the contrast response function for isochromatic flicker. As others have found, the shape of this function depends heavily on temporal frequency. At 18.76 Hz, we recorded reliable responses to cone-isolating flicker for all three classes of cones. At low contrasts, the cone response functions for all three cone classes are linear. We used a flicker-photometric paradigm to assess how signals from the three cone classes are combined. In this technique, the responses to various cone-isolating stimuli and combinations of cone-isolating stimuli are balanced against the responses generated by an interleaved reference stimulus. Two subjects gave differing results: for one subject the rate of combination was close to linear, in a second subject we observed strong spectral opponency between the long and middle wavelength sensitive cones.

646.9 DYNAMICS OF ADAPTATION TO CHANGING SPATIAL STRUCTURE IN THE TIGER SALAMANDER RETINA. S.M. Sinanakis*, M.J. Berry*, D.K. Watanabe*, W. Blair*, and M. Meister. 1Psych Dept and Medical School, Harvard University; 2MCB Dept, Harvard University; 3NEC Research Institute, Princesse, NJ.

The neural code employed by the retina is known to adapt dynamically to changes in the statistics of the visual scene, such as the mean intensity and temporal contrast. Here we focus on how the retina adapts in the spatial correlation length of the image. Isolated salamander retinae stimulated with flickering checkerboard patterns and ganglion cell spike trains were recorded extracellularly with a multielectrode array. The intensity of each checker was chosen randomly every 30 ms from a Gaussian distribution of fixed mean (photopic range) and root-mean-square contrast (24% of the mean). Every 100 s, the size of the checkers was changed, ranging from ~14° of a ganglion cell full field. We observed adaptation to these transitions in both the ganglion cell firing rate and the reverse correlation to the stimulus. The steady-state firing rates of ganglion cells depended on checker size; larger receptive field centers correlated with maximal responses for larger checkers. After a transition in checker size, the firing rate approached steady-state exponentially. When alternating between simulation, the rate of 25 out of 45 ganglion cells decreased with adaptation for both transitions; the decline ranged from 5% to 100% of the final value, with time constants clustered around 10 s. This suggests that there are at least two independent sites of adaptation in the retina. Because the stimuli all had the same local statistics, it is unlikely that either site is in the photoreceptors. Furthermore, changes in spatial correlation length also altered the temporal aspects of the neural code: reverse correlation showed that the response latency of both the center and the surround decreased for larger checkers.

Supported by a Pew Scholarship and a grant from the ONR to M.M.

646.10 NEURAL CODING IN THE LIMulus VISUAL SYSTEM. CL Passaglia*, FA Dodge, and RB Barlow. Institute for Sensory Research, Syracuse University, NY 13244.

The Limulus eye samples its visual world with a relatively small number of retinal receptors and optic nerve fibers (4000). Even so, environmental noise further limits the information capacity of the optic nerve, and yet male Limulus can detect females both day and night. Is the eye tuned to detect mates? We address this question by measuring the spatiotemporal transfer function (STTF) of the eye, defining the spectral properties of the environment, and developing a computational model of the eye to explore the information content of the optic nerve. We measured STTFs of single optic nerve fibers from submerged animals using an sinusoidal generator-recorder system (VENUS). We also recorded optic nerve responses from behaving animals in their natural environment. We then computed "neural images" for both binocular patterns and natural underwater scenes recorded with a camera mounted on the behaving animal. By analyzing the "neural images" and conducting parametric studies of the model eye, we studied the eye's tuning properties and its ability to cope with environmental noise.

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

NIH grants EY00667 and MH49741 and NSF grant BNS9309539.
ANATOMICAL IDENTIFICATION OF SHORT WAVELENGTH SENSITIVE (S) CONE INPUT TO H1 AND H2 HORIZONTAL CELLS IN MACAQUE MONKEY RETINA. A.K. Goodrich**, T.L. Chen and U. I. Gunten*. Department of Physiology F13, The University of Sydney, N.S.W. 2006, Australia.

In vertebrates horizontal cells have selective connections to cones of different spectral types. In primates, the presence of such selectivity remains controversial. In order to address this question anatomically, we studied the connections of H1 and H2 horizontal cells with photopic S cones in macaque monkey retina. Horizontal cells were labelled in two ways: 1) An axo-retinal wholemount preparation was used to inject horizontal cells intracellularly with Neurobiotin. 2) In paraffin-embedded fixed retinas horizontal cells were labelled with DiI. The fluorescent DiI label was photoconverted to a permanent reaction product (Sandberg, J.R., J. Cell. Biochem., 15:553, 1985). The retinas were then processed with antibodies against human S cone pigment (IH455, kindly provided by Dr. J. Nadanac). The following results were obtained. Labelled S cones made up 7% of the cone population and formed a regular array similar to that described previously (Dehmenstater et al., Science 213:1278, 1981). The entire cone including its pedicle was labelled, enabling us to investigate the contacts of S cone pedicles with labelled horizontal cells. Most H1 horizontal cells (34 of 41 cells) did not contact S cones. Labelled dendrites were seen passing over S cone pedicles but not contacting them. In those cases where H1 cells contacted S cone pedicles (7 of 41 cells) comparatively few (always less than 3) dendritic terminal knobs made these contacts. H2 cells (n=4) contacted all 5 S cone pedicles within their dendritic field. Dendrites of H2 cells tended to aggregate at the S cone pedicle. Contact was made by numerous dendritic terminal knobs, whereas contacts with undamaged pedicles were only sparse. Our anatomical findings suggest that H1 cells receive little or no input from S cones whereas H2 cells receive input from all cone types. Our results are in agreement with the recent physiological findings of Dyck and Lee (AVO Abstr., IOVS 36:10, 1995). Supported by the NHMRC and the Alexander von Humboldt Foundation.

THE LOW-FREQUENCY VOLTAGE NOISE IN THE ROD NETWORK OF THE DARK-ADAPTED FROG. K. Dippensemper** and T. Hirsuvuori. Department of Biometrics and Systematics, FIN-00144 Univesi Helsinki, 4Dept Physiology, FIN-90220 Univ Oulu, Finland and Research Center for Applied Information Sciences, Tokoku Univ, Sendai 980-77, Japan.

Previous studies (e.g. Bayler et al., J. Physiol 309, 191-212) have shown that isomerization events in isolated rods occur in the low-frequency spectral range. To see how these results correspond to the noise output in the intact rod network we studied the low-frequency voltage noise in dark-adapted Xenopus laevis (Rana ridibunda) rods and estimated the equivalent isomerization rates.

The study was carried out by intracellular recordings in completely dark-adapted (>1%) eyes. In addition we verified the pigment composition with HPLC. The IC recordings consisted of flash responses and 200 s noise periods in darkness and under a background of 1 isomerization per rod/s (=1 Ra/2s), in 10 cases in two temperatures. The spectral analysis was done with 1024 point FFT/Hanning windowing.

The visual chromophore was purely A. The amount of equivalent isomerizations in the voltage noise and in darkadapted (>1%) eyes. In addition we verified the pigment composition with HPLC. The IC recordings consisted of flash responses and 200 s noise periods in darkness and under a background of 1 isomerization per rod/s (=1 Ra/2s), in 10 cases in two temperatures. The spectral analysis was done with 1024 point FFT/Hanning windowing.


The response characteristics of the pigeon’s retinal ganglion cells were examined by direct intraretinal extracellular recordings. According to the theory of opponent color perception, the dark-red and phasic, were found. Phasic cells are mostly ON/OFF movement sensitive units, while tonic cells show predominantly exclusive ON or exclusive OFF responses, and lack movement sensitivity. Furthermore, phasic and tonic units have different chromatic properties. Phasic cells show wide action spectra, and 50% of them show OFF responses to the movement of pure chromatic edge in the long-short and long-middle spectral range. On the other hand, tonic cells have narrow action spectra, and 20% of them show well defined color opponent surround sensitivity. We also studied the spatial distribution in the visual field of these responses. Phasic responses can be found in the whole retina, but they are more abundant and dense packed in the lateral retina and the peripheral fovea. Tonic responses are almost exclusively present in the dorso-temporal retina. Accordingly, we think that the two kinds of ganglion cell responses contribute to the generation of perceptual properties that the frontal and lateral gazed of birds have.

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

The a-wave of the murine ERG reflects the activation phase of the rod photoreceptor response, whereas the photoreceptor response phase is obscured by other components of the ERG. We recorded corneal ERGs of CBA/CaJ mice, dark adapted for 2 hr and anesthetized with a ketamine-xylazine-urethane mixture. Whole-retina stimulation was provided by pairs of light flashes. The amplitude of the a-wave response to the second, saturating flash was used as a measure of the degree of rod recovery from the first. The entire recovery time course from flashes producing 300 - 20,000 photonisomerizations/rod (Rh*rod) was reconstructed by varying the intervals between flashes. The reconstructed responses were similar to those previously recorded from the photoreceptor layer of isolated rat retina. Over the intensity range 300-500 Rh*rod recovery half-times increased linearly with Rh*rod, with an apparent time constant of 0.3 s. (Supported by NIH EY-02660.)

DUPPLICITY WITHIN THE NOD MECHANISM OF RAT. P. Haaparanta, Physiol. Dept., Northeastern University, Boston, MA 02115.

Flicker studies on human revealed two separate rod pathways: a slow and a fast pathway (Conner, JPhysiol, 232, 82). Rod flicker threshold vs luminance functions showed a double branch and flicker frequencies of 14-15 Hz appeared inviable over a limited range of retinal illumination. In this range, a slow and a fast rod signal are thought to interfere destructively with each other and cancel flicker visibility (Stockman et al., JOSA, 91). We examined whether a similar duality of temporal function occurred in the rod system of rat. Electroretinographic (ERG) responses to square-wave flicker or to sinusoidally modulated light were recorded at the cornea of unethesized, male, albino rats. Results were as follows: (a) In a plot of CRF vs luminance, CRF increased to about 15 Hz when the luminance reached -2.3 log cd/m^2, then decreased to 11 Hz over the range of 2.0 to 0.8 log cd/m^2. Above -0.5 log cd/m^2 rod CRF rose again reaching 19 Hz at 1 log cd/m^2. (b) flicker amplitude vs luminance curves showed that, up to 0.5 log cd/m^2, response amplitudes to 3-5 Hz flicker increased monotonically. Amplitudes to 7-15 Hz flicker first increased then began to decrease at 2.0 log cd/m^2. Responses to 13-15 Hz flicker disappeared completely at 1.5 log cd/m^2 but reappeared at -1.0 log cd/m^2 when amplitudes for frequencies > 7 Hz began to grow again. Above this intensity response amplitudes to 17 and 19 Hz flicker were measurable. The phase of the responses to 13 Hz flicker at -2.0 log cd/m^2 was shifted by 180° relative to that recorded at -0.5 log cd/m^2. Our data suggest that there is a duality of temporal functions within the rod system of rat and it strongly resembles that of human.
64.2 CHANGES IN THE PUPILLARY LIGHT REFLEX OF THE RD MOUSE WITH AGE AND THE EFFECTS OF PHOTORECEPTOR TRANSPLANTS

S.K. Parpura, S.O. Whiteley, T.M.P. I. MacNeil and R.D. Land
Dept. Pathology, Institute of Ophthalmology, Bath St. London, EC1V 9EL, U.K.

The retinal degeneration (rd mouse) is a model for retinitis pigmentosa. It has a mutation in the β-subunit of cGMP phosphodiesterase which causes photoreceptor degeneration starting at day 8 and complete by 5 weeks of age. The rapid and total loss of the photoreceptors renders the model particularly useful for investigating the potential of transplanting photoreceptors. Although a number of groups have transplanted photoreceptors to the subretinal space (Gouras et al., 1992; Invest. Ophthalmol. Vis. Sci. 33:2792-2786) there are no published data on the function of such transplants. Using a compartmental pupillometry system we have studied the pupillary light reflex (PLR) of the rd mouse over time as a baseline for evaluating the function of retinal transplants in these animals. The amplitude, latency and latency of the constriction was analyzed at two different light intensities. The PLR was studied in rd mice at ages of 3 months, 6 months and 9 months. We found an increase in both the amplitude and latency of the PLR with time, at both light intensities. The response of 9 month old animals showed a greater amplitude and latency than the 3 month old of mice.

Retinal dissociates from newborn mice were transplanted successfully into rd retina. The anatomical evidence is presented. The responses recorded from eyes that had received transplants differed from those of control eyes.

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

64.3 RETINAL DAMAGE OF SOLAR ORIGIN IN A VANISHING SPECIES OF AMPHIBIAN, RANA CASCADAE: K. V. Flay, L. Bergeton, A. Blaustein, H. Hezek. Neuroscience and Behavior Program, UMSS Amherst, MA, U.S.

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

Quantitative histopathological analysis of the outer retina in three groups of feeding and non-feeding R. cascadae subjected to normal and UV-B levels in two different habitats, in the sun as compared with the shade, was conducted. Both retinas from 7 frogs in each group were evaluated using samples taken from superior and inferior retinal regions, embedded in epon, sectioned at 1μm thickness and stained with thionin for light-microscopy evaluation.

Results: A similar profile of histopathological features were observed both in R. cascadae and the experimental light-damaged R. pipiens. Abnormal decreases of melamin pigment, abnormal rod outer segments, reduced number of cones, and vacuolated inner segments. In R. cascadae, the inferior retina was substantially more abnormal than the superior retina. Relatively few abnormalities were observed in normal R. pipiens. These findings are consistent with the hypothesis that increased UV-B in solar radiation may be having deleterious effects in retina of amphibians and contributing to the worldwide "vanishing amphibian" crisis. (Supported by NSF Grant # 8601597 to K.V.F)

64.5 FELINE RETINAL DEGENERATION RESULTING FROM DIETARY B-ALANINE. H. Imaki, J.M. Messing and J.A. Sturrman. New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314

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

Light and electron microscopy examinations of retinas from these cats revealed that B-alanine ingestion accelerates the photoreceptor degeneration and elimination demonstrated previously in cats after a longer period of taurine deprivation. The degree and extent of degeneration resulting from B-alanine treatment was approximately proportional to the reduction in retinal taurine concentration, determined in these cats fed the B-alanine-supplemented diet. In this study, some cats fed the B-alanine-supplemented diet showed relatively normal retinal structure and the highest retinal taurine, others had reduced number of photoreceptors with markedly increased intensity and lower retinal taurine. Among the cats fed the taurine-free diets, one showed no remaining photoreceptors in any areas examined, and the lowest taurine diets were associated with varying degrees of retinal degeneration. In general, the most severe changes were noted in the nasal and optic disc regions, and the least in the inferior region.

64.6 INCREASED EXPRESSION OF RETINAL TIMP mRNA IN SIMPLEX RETINITIS PIGMENTOSA IS LOCALIZED TO PHOTORECEPTOR-RETAINING REGIONS. C. Jomar, S.E. Jones and M.I. Neil. Washington Retinitis Pigmentosa Society Laboratory, U.M.D.S., St Thomas Hospital, London, U.K.

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

Paraformaldehyde-fixed control and RP-affected human retinas were frozen in isopentane and 10 μm sections were cut and stained with hematoxylin and eosin. Using the derived cDNA probe, TIMP3 was hybridized with labeled sense and antisense riboprobes complementary to human TIMP and autoradiographed.

In control retina, no TIMP3 mRNA expression was detected. In contrast, in RP-simplex affected retina, a strong pattern of expression was observed. In regions lacking photoreceptors, expression of TIMP3 mRNA was localized particularly to the photoreceptor inner segments and the inner retina.

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

Supported by grants from the British Retinitis Pigmentosa Society.
A CHANCE IN THE NUMBER OF NADPH DIAPHORASE-STAINED RETINAL CELLS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT RETINA. B. Roudaut, S. S. Reis, R. Bajaj and P. Eleftheriou, Department of Anatomy and Cell Biology, University of Melbourne, Parkville 3052, Australia.

NADPH diaphorase (NADPH) is a nitric oxide synthase (NOS) which is found in association with the retinal vasculature in the rat retinal nerve. NOS is required for the production of nitric oxide (NO) a potent vasodilator and neuronal NOS may play a role in linking the control of the retinal vasculature and neural activity. Our results suggest that the number of NADPH-positive cells in the diabetic retina may be reduced due to a potential decrease in the expression of NOS activity.


Our purpose is to develop and evaluate a primate model for human retinitis pigmentosa (RP), using sodium iodate (IAA) to induce photoreceptor degeneration. Neel (1935) found that IAA induces a degeneration histologically similar to RP. We examined the ERG from a primate model in two macaque monkeys, which were followed for 16 and 19 months after acute systemic IAA administration. Visually guided behavior remained, and the effects in the two eyes were similar. A novel non-invasive quasi-focal cone ERG method (Wolf & Groom, 1995) indicated that IAA spares the function of macular cones only and that the foveal cone functions are lost by IAA than parfoveal cones. Over the course of several months cone ERGs decreased, and the fundus showed typical RP signs: vascular narrowing, wavy pallor of the disc, and retinal pigment epithelial and choroidal atrophy. Confirming Neel, we found the outer nuclear layer (ONL) to be devasated except in the central retina, where a gradient of damage extended from a relatively normal fovea to a monolayer of cone-like ONL, somata outside the macula. The inner retina was grossly normal. Also observed in all the IAA retinas was the occasional RP complication of tyrosin macular edema.

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

Wolf, M.J. & Groom, DH, ISCVM, June 2015. Supported by J. Epstein Foundation, Photoregiosis Inc., NEI EY02657, and RBP.

648.1 REVERSE CORRELATION MAPPING OF SUBTHRESHOLD SYNAPTIC POTENTIALS IN CAT PRIMARY VISUAL CORTEX. L. Graessle, V. Bresqueur, V. Frerichs, L. Bong-Graham, C. Monier (1), and G. Feurly (2), Institute Alfred Fessard CNRS (1), and Ecole Supérieure d'Electricité (2), France.

Extraocular analysis of cortical receptive fields (RFs) has revealed the existence of "unresponsive" surround regions by their modulatory effects on the cell's response to a stimulus presented within the minimal discharge field (MDF). Obtaining direct evidence for subthreshold excitatory and inhibitory regions extending beyond the classical RF requires methods for generating, recording, and detecting physiologically identified EPSPs and IPSPs. Simple RFs cells in area 17 of anesthetized cats were recorded using sharp intracellular and white-cell pulse electrodes while providing pseudo white noise input (small light flashes or bars flashed randomly in the visual field every 50ms). The following algorithms were used to detect occurrence times of PSPs in the waveform: (a) threshold-crossing of membrane potential, (b) threshold-crossing of waveform energy, or (c) correlation with the averaged baseline within a brief time window, and (d) prototypical and waveform-specific template matching to discriminate between fast and slow IPSPs and EPSPs, Ca"+" and Na"+" spikes. Event dissection window was prepared by stimulating ensemble to yield high spatial resolution IPSP and EPSP maps.

Subthreshold events show both excitatory and inhibitory regions extending up to nine times the area of the classical MDF. Map variance peaked when template matching was used (especially for inhibitory-like events). All excitatory profiles were consistent with stimulus-locked waveforms averaged at each spatial location for both types of stimuli. Moreover, the reverse correlation technique revealed inhibitory regions which were undetected using stimulus-locked averaging. The physiological nature of the subthreshold events detected was established from recordings at different membrane potentials and/or during QX 314 application. We thank Ralph Freeman for providing stimulus generation algorithms. This work was supported by grants to Y.F. from HFSF and Conseil de l'EsRonne.

648.3 THE CONTRIBUTION OF LGN M AND P LAYERS TO THE CONTRAST SENSITIVITY OF PRIMATE V1 NEURONS. J.D. Allison, P.M. Molnar, Y. Ding, J. Dicarlo, A.B. Bondi, and V. A. Casagrande. Dept. of Cell Biology, Psychology and Electrical Engineering, Vanderbilt University, Nashville, TN 37232.

Magnocellular (M) neurons in the LGN of primates, including bush babies, exhibit higher contrast sensitivity (i.e. lower contrast thresholds) and saturate under lower stimulus contrast than parvocellular (P) LGN neurons. The contributions of LGN M and P layers to M or P LGN neuron sensitivity to contrast sensitivity of individual V1 cells are unknown. To examine this issue we injected an injection/recording electrode containing 25 nm GABA in saline into either (a) the contralateral untreated M (layer 1 or P 6) or (b) the LGN of 8 bush babies. Multunit activity was recorded to identify the layers and locate the receptive fields. We then inserted a recording electrode into V1 and, after bringing both the cortical and LGN receptive fields, measured the contrast response functions (CRFs) of single V1 neurons using spatio-temporally optimized drifting sine-wave gratings presented to the contralateral eye. The CRF of each V1 neuron was repeated after blocking activity in either the M or P layer with an injection of GABA. When the M layer was blocked (n = 6), the average contrast sensitivity of V1 neurons was reduced from 2.82 (S.D. = 0.95, P < 0.05). When the P layer was blocked (n = 6), the average peak response amplitude at high stimulus contrast levels (i.e. 50%) was significantly reduced from 21.4 spikes/s to 8.3 spikes/s (f = 0.95, P < 0.05). The results indicate that the low contrast/highest sensitivity component of the CRF of V1 neurons P cells mainly contribute to the high contrast/low sensitivity component. These results suggest that the M layers of V1 neurons, Support by EY06410, EY03778, EY01778, and EY08126.

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

The magnocellular (M) inhibits the parvocellular (P) visual subsystem. We present VEP evidence that M/P interactions are subject to important temporal influences. Steady-state VEPs (2nd harmonic) were recorded (Oz to T3) in two experiments from 21 and 22 adults. Stimuli subtending 14 deg were horizontal gratings differing in spatial frequency and contrast (M = 0.06 c/d and 17c/d at 15 Hz, P = 5.00 c/d with 848c/d at 3 Hz). In Exp. 1, M and P stimuli were simultaneously presented once every 6.8 s (or 2.85, P < 0.05). When M layer was blocked (n = 6), the average peak response amplitude at high stimulus contrast levels (i.e. 50%) was significantly reduced from 21.4 spikes/s to 8.3 spikes/s (f = 0.95, P < 0.05). When the P layer was blocked (n = 6), the average peak response amplitude at high stimulus contrast levels (i.e. 50%) was significantly reduced from 21.4 spikes/s to 8.3 spikes/s (f = 0.95, P < 0.05). The results indicate that the low contrast/highest sensitivity component of the CRF of V1 neurons P cells mainly contribute to the high contrast/low sensitivity component. These results suggest that the M layers of V1 neurons, Support by EY06410, EY08126.
648.5 EFFECTS OF ADAPTING ON SPATIOTEMPORAL RECEPTIVE FIELD STRUCTURE IN CAT STRIATE CORTICAL SIMPLE CELLS. A. L. Salt*, Dept. of Neurology, Harvard Medical School, Boston, MA 02115.

Adapting changes timing, as well as amplitude (Vas. Neurones. 12:191-205, 1995). Simple cells responded to drifting gratings are delayed at onset, but unaffected at offset. By examining receptive field structure in control and adapted states, we have gained some insights into the origins of these specific aftereffects. These studies also allow us to examine the role of different components of response properties.

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

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

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

Supported by BNS-9212493, ES-10826, and EY-08098.

648.7 A ROLE FOR GABA-MEDIATED INHIBITION IN GENERATING THE SPATIOTEMPORAL STRUCTURE OF SIMPLE CELL RECEPTIVE FIELDS. J. M. Bisley*, K. A. Drislane, Dept. of Neurobiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Many simple cells possess gradients of response timing across their receptive fields. This property, known as spatiotemporal receptive field (STRF) organization, and it is correlated with cells' direction selectivity (DS). The mechanisms underlying DS-orientation are unknown; they may reflect inputs from the lateral geniculate nucleus, the striate cortex, or a combination of both. In the present study, we examined the role of intracortical inhibition.

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

Of 11 direction-selective cells with S-T-tuned receptive fields, 7 showed NMB-induced reductions in DS in S- and ST-scale. Response timing became more uniform across their receptive fields. For these cells, changes in the two measures were correlated (r=0.87; p<0.01). In contrast, the other 4 direction-selective cells showed little or no change in DS despite NMB-induced increases in their visually driven activity. S-T orientation in these cells was affected more variably. These data show that S-T structure and DS covary in single cells; this extends previous observations based on comparisons among populations of cells. In addition, the data reveal that GABA-mediated inhibition can act to create direction selectivity through its impact on S-T structure. However, heterogeneity appears to exist among simple cells in the dependence of their direction selectivity on this inhibition.

Supported by EY04459 and EY08096.

648.8 SPATIAL SUMMATION PROPERTIES OF THE MACAQUE V1 NEURONS IN THE RETINOGENICULATE REPRESENTATION OF THE BLIND SPOT. H. Kangas, M. I. Murakami, Dept. of Neuroscience, Research School of Physical Sciences, Australian National University, Canberra, A.C.T., Australia.

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


The visual system utilizes binocular disparity to obtain information about the relative depth of objects in space. Most cells in the striate cortex are sensitive to binocular disparity, suggesting that these neurons form part of the mechanism that steroepsis (Barlow et al. 1967; Pettigrew et al. 1968). It has been suggested that binocular disparity is encoded by spatial offsets (position disparity) of left and right eye receptive fields (RFs) that have the same spatial phase. It has also been suggested that binocular disparity can be encoded through differences in RF phase between the two eyes without position disparity (Fremouw & Ohtaka, 1990; DeAngelis et al. 1991). These two questions as to whether simple cells encode RFs through position disparity alone or through both position and phase disparities to answer this question, we simultaneously map RFs of multiple simple cells in anesthetized cats to obtain phase disparity for each cell as well as position disparity between the cells. We then determine the relative contributions of phase and position disparities to binocular disparity of the tuning cells. Dynamic noise patterns generated according to binary in sequences are presented over the RF locations on two graphically identical, one for each eye. Cells were also sampled across different conditions and the enhanced responses were observed along the midline of the strips. This increases the robustness of the measure and adds to the stability of the analysis. Our group has correlated the results of responses to the cell patterns are cross-correlated with the stimulus sequence used to obtain the RF maps. These maps are then fitted to a Gaussian function to estimate phase and center coordinates of the RFs. Phase and relative position disparities are obtained from the estimated parameters.

We find that the disparity of some simple cells exhibits a preference for position as well as in phase. Although the contribution of position disparity to the disparity preference of some cells is substantial, position disparity is generally limited to the extent that it is equivalent to a 90° phase disparity. Therefore, we conclude that these simple cells encode binocular disparity through both position and phase disparity alone may not be sufficient to encode large disparities. (EY01175)
648.11
COMPARISON OF THE CLASSICAL AND THE INTERCULAR SUPPRESSIVE RECEPTIVE FIELD USING RECEIVER OPERATOR CHARACTERISTIC ANALYSIS REVEALS A CLOSE SPATIAL CORRESPONDENCE IN ANAESTHETISED CAT. Toby C. B. Freeman, Frank Sengpiel, and Colin Blakemore. University Laboratory of Physiology, Oxford OX1 3PT, U.K. and 1Bristol Eye Hospital, Bristol BS1 2LX, U.K.

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

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

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

Supported by MRC and Oxford McDonnell-Paw Centre for Cognitive Neuroscience

648.13
SPATIAL PHASE GRADIENTS IN NECOTROICAL EEGLS GIVE MODAL DIAMETER OF "BINDING" DOMAINS IN PERCEPTION. W.J. Freeman, N. Barrie, M. Leff, and R.X. Tang. Dept of Molecular & Cell Biology, Univ. of California, Berkeley CA 94720

Spatial patterns of EEGs were derived from 8x8 epidural electrode arrays (0.5-0.8 mm spacing) on the olfactory bulb (OB), prepyriform cortex (RPC), and the visual, auditory and somatic cortices of rabbits. Phase was measured for components of the aperiodic wave forms in segments (64-121 ms) with Am-FM modulated Fourier components. Phase gradients in the OB had the form of a cone, for which the location and signs of the apexes (maximum lead/lag) varied randomly. Phase velocity converged to 1.80±0.43 in m/s, the estimated conduction velocity of mitral-tufted axon terminals. Most (80%) phase gradients in OB had the conduction velocity of olfactory tract (2.6 m/s). Others were endogenous gradients at 1.0 m/s for activity in low gamma and high beta ranges (20-60 Hz). Neocortical velocities had skewed distributions on frequency, from 2.0 m/s at 12 Hz to 3.5 m/s at 48 Hz. Commonality ("phase locking", "binding") was defined as oscillation within the half power range (± cos 45°). An invariant estimate emerged on multiplying the phase velocity in mm/s by 0.25 cycle in ms = 21.0±0.5 mm for modal diameter of most neocortical domains, but 6.12±0.5 m/s for some in somatic cortex. This is consistent with EGCo data from exposed hippocampus (2± cm) and with cat unit recordings (for example, at 40 Hz the estimated lag across 7 mm is 0.55 ms).

648.15
SYNCHRONIZATION OF SINGLE UNIT SPIKE TRAINS IN CAT VISUAL CORTEX IS STIMULUS DEPENDENT W.A. Freeman, W. Seger. Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt, F.R.G.

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

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

648.16
INTERHEMISPHERIC SYNCHRONIZATION IN CAT VISUAL CORTEX IS COMPARABLE TO SYNCHRONY WITHIN HEMISPHERES. A.K. Egbert; F. Kugel, P. Burget, M. J. M. Mant and W. Singer. Max-Planck-Institut für Hirnforschung, Deutscherstr. 46, 60528 Frankfurt, Germany

Recent studies of neuronal interactions in the visual cortex indicate that synchro nizing firing of neurons may be relevant for the integration of distributed responses into coherent repre sentational patterns. One important step in validating this hypothesis is the demonstration that synchronization occurs also between the two different cerebral hemispheres. Several years ago, we have reported the existence of such interhemispheric neuronal synchrony in cat visual cortex. However, recent progress in the understanding of cortico-corti co-synchronized activity in general has led us to believe that interhemispheric synchrony in greater detail to facilitate comparison with the synchrony observed within each hemisphere. Multitrial and field potential responses were recorded simultaneously from left and right hemisphere. Our results indicate that synchronization of field correla tions with a precision in the millisecond range can be observed between neurons in left and right visual cortex. On average, the synchronization occurred within the phase lag. The strength of the interactions was comparable to that observed within each hemisphere and was dependent on the overlap of the receptive fields but not on differences in the neurons’ preferred orientations. In synchronized correlations was almost always associated with an excitation mediated modulation of the responses at frequencies in the range of 30-80 Hz. Similar to the synchrony observed within hemispheres, the interhemispheric interactions were not consistent across different visual stimuli. Synchro nity was weak for incoherent stimulus arrangements and was higher for stimu li with similar orientation or direction of motion were used to coactivate different cell groups. The interactions were completely abolished in cats where the corpus callosum had been sectioned prior to the physiological experiment, demonstrating that the syn chronization is cortical by its current output from subcortical structures. Together, the data are compatible with the hypothesis that synchrony may be exploited to establish relationships between responses of spatially distributed neurons, synchronization across the hemispheres then serving for the binding of signals arriving from different visual hemifields. (AXE acknowledges support by the Heisenberg Program of DFG)

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468.17

We studied visual temporal coding by investigating the interneuronally generated signal correlations of neurons activated by the same visual stimulus. Single (SUA), multiple-unit (MUA), and local field potentials (LFP; 10-140 Hz) were recorded in the primary visual cortex of an awake macaque monkey by a linear array of 7 fiber-electrodes and analyzed by cross-correlation methods. During a fixation task the RFs were stimulated by a binocular grating (switched on at preferred orientation and spatial frequency, stationary for 1.7 sec, then drifting at a local temporal modulation of 2.3 Hz). Classification of neurons into simple or complex (classical test of spatial-temporal modulation) did not reveal the separable classes as in anaesthetized animals. Nevertheless, we divided the distribution into simple- and complex-like cells according to their depth of modulation. Their response latencies to the onset of the grating were not different, having a broad temporal dispersion with groupings around 50, 100 and 143 ms (N=72). Activities were typically non-rhythmic before and during the rising phase of the transient response to stimulus onset. During later response epochs, including stationary and slow movement stimulation, fast oscillatory responses were observed. These analyzed the neural signals (15-400 Hz). Both, phasic and rhythmic response epochs revealed cross-correlation peaks close to slow delay (slow ms) including SUA-LFP, MUA-MUA and MUA-LFP correlations at the same and different recording sites (cortical distance: 3±4 mm). The SUA-LFP correlation peak delay differences of stationary and moving recordings were significantly less than zero (p<0.01) and showed slightly different values for simple- and complex cells (C: -0.95±0.20 ms, N=188/5-0.34±0.32 ms, N=29). The present results support previous observations of cortical temporal coding around zero delay. Near-zero correlation was present independent of onset response latency and P50-frequency, non-rhythmic and rhythmic epoch, small and large cortical separation, simple and complex cell, MUA and LFP. However, merging cross-correlations over response time revealed other complex temporal patterns depending on neuron and stimulation. (Supported by DFG EZ556 and 537 to RE.)

468.19
VISUALLY INDUCED GAMMA BAND RESPONSES IN HUMAN EEG
M.M. Müller, J. Bosch, T. Elbert*, J. Riera, M. Valdes-Sosa, and B. Rockstroh
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On the basis of animal studies, gamma-band oscillations have been proposed as a possible fundamental physiological mechanism solving the binding problem. In contrast to evoked gamma-band responses (GBR), induced GBRs are neither field nor phase locked to the stimulus onset. Averaging of trials most certainly will result in the disappearance of GBRs in the grand mean. In addition, the anatomical location of Area 17 may lead to a neutralization of electrical dipoles when visual full field stimulation is carried out.

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

Supported by the Deutsche Forschungsgemeinschaft Mu 972/0-1

469.1
MAXIMIZATION OF INFORMATION IS CONSISTENT WITH THE ORGANIZATION OF VISUAL CORTEX. B.J. Richmond* and O.X. Jin, Laboratory of Neuropsychology, NIH, Bethesda, MD 20892.

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

469.2
TIME SCALES IN THE INFORMATION PROCESSING DYNAMICS OF PRIMATE STRIATE CORTEX COMPLEX CELLS. G.K.J.A.* T.J.Gawne*, I.Hertz, and J.Richmond, Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda MD 20892, and NORDITA, DK-2100 Copenhagen, Denmark.

The time scales associated with the dynamics of neural information processing are not well understood. We measured information transmission in the responses of V1 complex neurons in awake monkeys to sequences of two-dimensional gratings presented as a moving random and regular pattern. The speed of the moving was varied, the duration of each pattern in the sequence ranging from 10ms to 128ms.

In 7 monkeys the fixed sequence were quite stereotyped, with pulses related to the pattern change often appearing to be aligned across trials, as reported by Bair and Koch for MT neurons. For the random sequences, we estimated the information transmitted about stimulus pattern using a neural network. We found: (1) The stimulus-related information rises as the duration of the patterns in the sequence increases. (2) Information begins rising about 40ms after the stimulus appears. (3) Information rises steeply during the next 40ms and then continues to rise more gradually than at least another 90ms. (4) The duration of the information rise is never longer than the length of the presentation period for a pattern. (5) The rate of information transmission peaks between 10 and 20 Hz/sec, and remains high for a substantial proportion of the stimulus duration. Points (3) and (5) together suggest that the response carries a large amount of redundant information. There is information about the preceding stimulus in the visual response to the current stimulus, i.e., there is a short memory of the preceding stimulus; this memory lasts for at least 30 ms.
469.3
HOW MUCH INFORMATION IS CARRIED BY CORRELATED NEURONS? P.E. Latham, G.X. Jin, T.J. Gawne, and B. J. Richmond. Lab. of Developmental Neurobiology, NICHD, and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892. Experimental results from V1, IT and MT indicate that neuronal responses are correlated ($\rho_Y \equiv$ signal correlation $\approx 15\%$ and $\rho_N \equiv$ noise correlation $\approx 5\%$). How much do these correlations affect the amount of information a population of M neurons can carry about a stimulus? For a Gaussian distribution of neural and noise, and a signal to noise ratio of $S/N$, the mutual information, I, is given by

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

As long as the signal is not totally correlated ($\rho_N < 1$), the information scales as the number of neurons, M. Correlations in the signal induce correlations in the response, so the information scales as $M^2$ even when the neurons are correlated. For a completely correlated signal ($\rho_N = 1$) and uncorrelated noise ($\rho_N = 0$), the information scales as $\log(M)$. Finally, for a completely correlated signal ($\rho_N = 1$) and nearly uncorrelated noise ($\rho_N > 0$), the information asymptotes to a constant. In ourposter we extend these results to non-Gaussian distributions, and discuss ways to compute information from neuronal populations in realistic experiments.

469.4
COMPUTING MEAN FIRING RATES OF ENSEMBLES OF REALISTIC NEURONS. A.G. Luquet, E. Todorov and D.C. Somers2. MIT. E25-147A, Cambridge, MA 02139. Realistic models of neural circuits typically are too complex to be analyzed exactly and have too many parameters to be fully explored in computer simulations. Thus, methods for characterizing the collective dynamics of such systems are needed. Here, we develop a technique for approximating the mean firing rate of highly interconnected neuron groups that receive Poisson input. This technique applies to single compartment neurons with realistic synaptic and intrinsic currents. Assuming only that conductances equal their averages, we derive a nonlinear equation relating the mean firing rate to the cellular and network parameters. This approach extends to the analysis of multiple neuronal groups, for which a system of nonlinear differential equations, which exactly match the current system of analysis, can be used. We compare our analytical results to simulations and observe a close fit for most parameter settings. In a system with 2 excitatory and 2 inhibitory populations, there are over 250 cells, the approximation error is less than 2%. The accuracy of mean firing rate calculations depends on proper estimation of the afterhyperpolarization potential. Unlike most related techniques which assumes a constant rest potential, our method computes this as a function of input. The current form of our analysis is inaccurate only near threshold input levels where conductance variability significantly affects firing rate. We are presently extending the approximation technique to take this variability into account. Computing the mean firing rate with our technique reduces to solving a system of nonlinear equations, which is much faster than full simulation. In addition to guiding the search for suitable model parameters, our technique can yield analytical insights not available through simulations. For example, we can estimate the sensitivity of the mean firing rates to changes in model parameters. In addition, the system of equations can be solved directly to find parameter settings that achieve a desired set of mean firing rates.

469.5
SUBCORTICAL CONTROL OF SYNCHRONIZATION OF CORTICAL ACTIVITY: A MODEL. P. König, P.F.M.J. Vreeswijk1. The Neurosciences, University of San Diago, CA 92108. A large body of experimental results suggest that the synchronization and desynchronization of neuronal activity is an important feature of cortical dynamics. These processes may serve to segregate and segregate populations of neuronal units. A consequence of this hypothesis is that, not only the level of neuronal activity, but also the level of coherence in the cortical network is regulated.

Here a model of the interaction of cortical areas with subcortical structures is presented, which addresses this issue. In the cortical network the distribution of neurons along the primary hemifield is captured by taking into account their future electronic length. This parameter specifies the size of the set of effective synapses. The electronic length is influenced by modulatory transmitters released by the afferents of subcortical structures, which are modeled to be very sensitive to synchronous input and are assumed to perform a spatial integration of cortical activity, thus forming a regulatory system for the level of cortical synchronization. These general principles ($\rho_N = 1$) apply to a model of the visual cortex demonstrating the adjustment of the interaction of cortical neurons to the degree of coherence present in the visual stimulus. Furthermore, this mechanism allows the regulation of the spatial scale of cortical processing. These results are in accordance with recent experimental observations (Mink et al. 1994, Neuroscience Abstracts), which demonstrate the involvement of the parabrachial nucleus in the regulation of cortical synchronization.

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

469.6
SINGLE UNIT RECORDING CAPABILITIES OF A 100 MICROELECTRODE ARRAY. C. T. Nordhausen, E. M. Maynard, P. J. Reusch, R. A. Newton. Moran Laboratories, Department of Bioengineering, University of Utah, Salt Lake City, Utah 84112, USA. Recently, the focus of many cortical electrophysiological studies has switched from the conventional single microelectrode approach to multielectrode investigations. This has been the result of the interest in the parallel processing aspects of cortex, and the spatiotemporal firing patterns of neurons. We have developed a three dimensional silicon electrode array, the Utah Intracortical Electrode Array, which provides 100 separate channels for neural recording in cortex. In order to demonstrate the efficacy of the electrode array for intracortical recording, we have quantified various aspects of both acute and chronic recording capabilities. Our preliminary results from acute preparations indicate that 60 to 70% of the electrodes yield evoked neural activity. Histology indicates that the majority of the remaining electrodes do not yield neural activity because they are positioned over a sulcus. The amplitude of the responses recorded with the array range from 40 to 180 $\mu$V with background noise on the order of 10 to 15 $\mu$V. Although the signal to noise ratio of these recordings is not as high as in the case of single microelectrodes, we are able to identify, on average, 3.5 units for each electrode that has recordable activity using time and amplitude window discrimination techniques. We have identified and tracked the same units for up to 4 weeks postimplant in ongoing chronic implantations. These results indicate that the electrode array provides a stable substrate for long term neural recording in cortex.

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

469.7
OPTIMAL POPULATION CODES FOR A FUNCTIONAL ANALYSIS OF VISUAL CORTEX DYNAMICS. M.A. Giese, A.C. Ahkavan1, W. Elhage1, D. Jancke, A. Steinheide, H.R. Düuir, G. Schoener, Institut für Neuroinformatik, Ruhr-Universität, Bochum, Germany and CNRS-LNC, Marseille, France. Application of population codes for a simple reconstruction of the stimulus or effective response data does not reveal much insight in the functional mechanisms in the neural substrate. We redefine population code as a class of algorithmic schemes projecting a set of measured neural activities onto one or more interesting parametric dimensions. Spatiotemporal coding by a task or stimulus makes collective neural response data useful interpretable.

Regarding population coding as a mathematical estimation problem we construct an optimal population estimator for the neural activity distribution over an abstract parameter space. We use a linear population estimator with well defined statistical properties derived by generalization from the optimal linear estimator described by Salinas & Abbott (J. of Comp. Neurosci. 1: 49, 1994). Estimated activity distributions from cat primary visual cortex can directly be compared to predictions derived from a neural field model on dynamical spatio-temporal interactions in the visual field. The model reproduces psychophysically observed attraction and repulsion effects. It yields predictions for the activation dynamics in the underlying neuronal ensemble, which are specific for the functional properties of the neural substrate.

Supported by Studienstiftung des deutschen Volkes, Graduiertenkolleg KOGNET, and DFG, Di 334/5-1 und Scho 338/4-2.

469.8
PRESERVED MOTION DISCRIMINATION IN MONKEYS WITH EARLY LESIONS OF STRIATE CORTEX. T. Moore, A.B. Repp, H.B. Rodgers1, C.C. Gross. Department of Psychology Princeton University, Princeton, NJ 08544. The ability to discriminate visual stimuli on the basis of presence or direction of motion has been reported to survive damage to striate cortex in humans. In contrast, we previously reported (Moore et al., JNS Abstr 12: 1801, 1994) that monkeys with unilateral striate lesions could not distinguish direction of motion in small (5 deg. diameter) dynamic random dot displays presented in the hemifield contralateral to the lesion. To address the possible role of stimulus size and retinal position in the residual visual capacities following striate cortex damage, we tested the ability of 3 monkeys with unilateral striate lesions made in infancy (5-6 weeks) to discriminate direction of motion in considerably larger (15 deg. diameter) displays of 0.5 deg. resolution centered at 13.5 deg. on the horizontal meridian. Magnetic resonance imaging indicates a complete lesion in one subject and only limited sparing of the peripheral field representation in the other two. A go/no-go paradigm using a saccadic eye movement response was employed to test the monkeys' ability to discriminate upward from downward motion at 4 and 20 deg/sec. All monkeys learned the tested discriminations in both hemifields. The results suggest that in monkeys with striate cortex damage, some motion discrimination capacity is indeed preserved, at least after early lesions. Supported by NSF BNS-8910743 and NIH MH-19420.
649.9

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

649.11

Areas of the striate and extrastriate cortices which are responsive during a contrast scaling task were identified using functional magnetic resonance imaging (fMRI) techniques. The results of the per cent contrast and the magnitude of the fMRI signal was examined.

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

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

This work was supported by EV10294 (to EAD) and the Chapman Foundation of Milwaukee (to MRB).

649.13
CHANGES IN REGIONAL CEREBRAL BLOOD FLOW BY FLASH AND PATTERNED VISUAL STIMULATION IN MONKEY VISUAL CORTEX. K. Imanari*, K. Ono†, H. Tsukada†, Y. Watanabe†, T. Shinomizu†, K. Ono†, H. Tsukada†, T. Kakuchi†, and Y. Watanabe†, †Subfemtomole Biorecognition Project, JRDC, Osaka 565, ‡Osaka Bioscience Institute, Osaka 565. †Central Res. Lab. Hamamatsu Photonics K.K., Shizuoka 434, Japan.

We studied changes in the regional cerebral blood flow (rCBF) induced by visual stimulation using H215O and positron emission tomography (PET). Averaged subtraction images of different experimental conditions were made and signals were superimposed on the magnetic resonance (MR) images for statistical evaluation.

We found that (1) it was more difficult to obtain significant signals in the visual cortex under anesthetized and paralyzed condition than awake condition, (2) larger signal was obtained in binocular stimulation than monocular stimulation, (3) flash stimulation induced large signals in the visual cortex as moving, rectangular stimulation, (4) activity in the frontal region, including the cingulate cortex was decreased by visual stimulation, (5) visual stimulation applied to the deprived eye of monkeys that had been suffered monocular deprivation over the sensitive period still induced some responses in the visual cortex. These results show not only dynamic change in visual stimulation but also dissociation between the generation of action potential and rCBF-change in the visual cortex.


649.10

Human spatial sensitivity depends on the temporal frequency of the stimuli. At high temporal frequencies (TF), sensitivity to sinusoidal gratings is best below 3 c/deg while at low TF, sensitivity is best between 3 and 6 c/deg. In addition, sensitivity to high spatial frequency (SF) gratings decreases with eccentricity. We applied measured activation in human striate cortex (V1) to examine the neural correlates of pattern vision. We measured blood oxygen level-dependent (BOLD) fMRI signal in V1 while subjects viewed counterphase sinusoidal full and sine-wave gratings. We used a 2*2-weighted gradient echo spiral sequence (1.5T, 78.78x5mm voxel size, TR=75ms, TE=40ms, FA=23deg) and a cross-correlation analysis. Our results were consistent with psychophysical measurements. Low SF gratings produced best correlations with the stimulus at high TF while high SF gratings produced best correlations at low TF. Posteriors regions of V1, close to foveal representations, were primarily activated by high SF/low TF stimuli. Anterior regions of V1, corresponding to periphery representations, were primarily activated by low SF/High TF stimuli. These results may be a consequence of variations in receptive field size or a change in the relative amout of parvocellular and magnocellular input along V1.

Supported by Stanford OIT.

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

649.14

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

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To examine the idea that the turtle dorsal cortex may be important in the detection of movement, visually-evoked primary activity in the cortex was monitored using combined multi-site optical recording and video imaging techniques. The image at left shows the outline of a 464-element photodiode array superimposed on the unfixed cortical sheet using an isolated eyechip preparation of the turtle. Paramezymes: "Mov- ing stimuli" were generated using a matched-pair of laser diodes (3 mW, 670 nm) that delivered two light spots (200 ms duration) under computer control. Optical signals at retinotopic locations (stimulus block to grid) is longer (as when visual stimulation was switched between a reversed and a caudal located spot (100 ms)) that the responses observed when both light spots were delivered at the same visual location (thin traces).

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

VISUAL CORTEX: STRIATE VIII


Firing arising from the brain may provide challenging input to the lateral geniculate nucleus (LGN), while the visual cortex receives a cholinergic input from the basal forebode. Recent evidence has shown that nico-tinic acetylcholine (NOS), synthetic enzyme for nicotine (NO), is co-localised with ACh in both pathways. We have previously shown that NO is necessary for the transfer of visual information in the LGN, gating visual input by an action involving NMDA receptors. Here we tested these recently established results in the primary visual cortex of the turtle.

Experiments were carried out on paraplegic, anaesthetised adult cats (halothane 0.1-0.1% in NO and 70:30% (v:v) Sevoflurane 100 mg kg-1 h) Severe barrel depression were used for extracellular recording and iontophoretic injection of drugs. The effect of application of L-Arginine (NOS- nitric oxide synthase inhibitor) and L-Arginine (L-Arg, the physiological substrate of NO) was tested on spontaneous, visual and NMDA/LGMDA evoked responses. In 14/40 cells application of L-Arginine produced a decrease both in spontaneous and visual responses, without changing visual specificity. This effect was reversed in 11/14 by co-application of L-Arginine itself without effect and is therefore consistent with our data in the LGN. In 16/40 cells application of L-Arginine abolished cell responses, even using very low ejection currents. This effect was unaltered by L-NOG in any case. 25% of cells were unaffected by either drug, and both were abolished in combination. There was no clear relationship between the effect seen and either cell type or laminate position. In a different block of experiments, we have observed that ejection of L-NOG essentially suppressed both NMDA and AMPA responses in 40% of cells.

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

References

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We were interested in effects following from the Group 1 phosphoinositol linked metabolic receptors (mGluRs) in the visual cortex, and particularly actions that derived from modulation of the potassium channels underlying the M-current. Indeed, such actions provide a means of local control of excitation superimposed on cholinergic influences in the neocortical tissue. To this end we used microiontophoretic techniques to explore the action of a range of mGluR agonists on the visual and drug driven responses of cells in layers II-IV of the cat primary visual cortex. To our surprise we saw only a few examples of the type of facilitatory effects previously associated with the cholinergic modulation of the M-current (Silbido and Kemp 1983, J. Physiol. 252:287-304; Murphy et al. 1993, J. Neurophysiol. 69:1465-1476) and these were on cells in layers V and VI.

The predominant effect (90% of the cells) was a selective suppression of visual driving. Thus for example iontophoretic application of the multiple mGluR agonist 15,38-ACPD depressed or blocked the visual response of cortical cells to an optimally oriented moving bar, but did not influence the responsiveness of the cells to iontophoretic pulses of NMDA or AMPA. Thus the post synaptic excitability of the cells to the agonists acting at the postsynaptic glutamate receptor was not changed whilst the visually driven response was. This effect seems most logically explained by a presynaptic effect mediated via Group 2 mGluRs. We observed it in cells throughout layers II-VI and its prevalence suggests that presynaptic control of the transfer of visual information may play an important functional role in the microcircuity of the visual cortex.

650.7 DISTRIBUTION OF ADENOSINE A1 RECEPTORS IN PRIMARY VISUAL CORTEX OF MONKEY. S. Larochelle, J. Guimond and A. Chaudhuri. Dept. of Psychology, McGill University, Montreal, Canada.

The adenosine A1 receptor has been implicated in a variety of physiological functions, including inhibition of neurotransmitter release. Adenosine binds to A1 receptors with high affinity and inhibits adenosine cyclase. The distribution of this receptor in visual cortex has been poorly documented.

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

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

Supported by grants from MRC (MA-126085) and NSERC (GCI015482).


Although receptors for a variety of neuromodulators including serotonin (5-HT) and adenosine (A1) are present in the visual cortex, their physiological actions have mostly been inferred from studies in other preparations. In order to study these effects directly, we made whole-cell voltage and current clamp recordings from primate visual cortex in layers 2/3 and 5 of 5 primary visual cortical slices, under visual control, using infrared videomicroscopy. A1 receptors (10-100 μM) reduced the frequency of spontaneous EPSCs from 1.8 to 3.3 Hz. A2A receptors caused an apparent change in their amplitude. EPSCs evoked by visual intracortical stimulation were reduced by 40-70%. For stimuli which evoked minimal (apparently unitary) responses, A2A dramatically increased the failure rate. ADE also enhanced the synaptic facilitation and decreased the synaptic depression evoked by brief stimulus trains (5 ± 20 Hz). Effects of 5-HT (10-500 nM) on the specific responses. CS2 have found the A2A receptors to be localized to discrete layers of the visual cortex. These findings, together with the known effects of NO and GMP in other brain regions, suggest an important role for the NO/GMP system in visual cortical function. Supported by grants from the NIH.

650.9 VISUAL DEPRIVATION DOES NOT ALTER MUSCARINIC RECEPTOR PROTEIN DISTRIBUTION IN AREA 17 OF RHESUS MONKEYS. M. Yanes, J. Tipton, H.D. Reis, D.B. Rye and A. I. Laven. Yerkes Primate Center and Dept. of Neurology, Emory Univ., Atlanta, GA 30322.

Acetylcholine has been implicated in the normal function of visual cortex and in ocular dominance plasticity. We used anti-α1 muscarinic cholinergic receptor proteins m and m to investigate the expression of these proteins in area 17 of a normal rhesus monkey and of 2 monkeys raised with a black contact lens on one eye from birth and 2 monkeys raised with a black eyewall sleeve as adults. In area 17 of the control brain, immunoreactivity of both proteins was distributed in a complex laminar pattern. Adjacent choline oxidase immunoreactive sections were used to confirm assignment of immunoreactivity to various layers. m was detected in layers 6 and 2/3, followed by layer 5. Layer 4 did not contain intervening m. A2A receptor activation with nicotine produced a similar change in the distribution of m. m was detected in neuronal cell bodies and neuronal processes. m antibody labeled mostly process structures. In area 17 of visually deprived monkeys, the distribution of the 2 receptor proteins appeared normal, except that in one iduedured monkey a thin band at the 4CS border reacted more densely with m than m. Thus these proteins seem to be insensitive to monocular elimination of visual input via occlusion from birth and to long-term elimination of a particular visual input imposed on the adult visual system.

Supported by NIH grants EY07937, R01865, and NS30454.

650.10 NEUROCHEMICAL ORGANIZATION OF MACAQUE STRIATE CORTEX: CORRELATION OF CYTCHOME OxIDASE WITH NOS, NADPH DIAPHORASE, NMDAR1, AND NA+ K+ AT PASE. M. Wong-Riley*, W. Liebl and Z. Huang. Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Previous studies indicate that a) chymotryptic oxidase (CO-I) rich puffs in the macaque striate cortex sustain a higher level of maintained neuronal activity than interpuffs, b) neuronal and synaptic properties differ between these two components, and c) asymmetric glutamate (GLU) immunoreactive synapses predominate in puffs. A major Glu receptor family is NMDA, which is implicated in the stimulation of Glu oxidase (NOS) and the production of NO in puffs. At the EM level, the density of NOS-positive immunogold particles was significantly higher in CO-I-rich type C cells than in the other cell types of puffs. Thus, our results are consistent with an excitatory synaptic circuitry in puffs that involves glutamate and NMDA receptors, as well as nitric oxide in selective cell types. The excitatory synaptic activities are likely to impose a higher energy demand in puffs than in the surrounding interpuffs. Supported by NIHY05439 and NS18122.
650.11

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

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

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

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While intracortical inhibition clearly has a role in generating orientation selectivity in visual cortex, how the balance of excitation and inhibition regulates orientation specificity remains unknown. To examine the issue, we injected GABA into a column of known orientation preference and imaged intrinsic signals to ascertain changes in orientation strength and preference in the surrounding cortical region. At the maximal concentration used (1.0M, 9%6) we were able to affect sites up to 1.5 mm distant from the injection. Sites with similar orientations to that at the injection showed a shift in orientation preference away from the injection site orientation. Orientation preference at the injection site changed up to 45°, although general activity levels were not strongly affected. These effects were reversible. Surrounding columns of orthogonal orientation, as well as more distant columns of all orientations showed no obvious changes during GABA application. Changing the amount of GABA ejected produced changes in the magnitude of the orientation shift, and the extent of cortex involved. While these results do not favor a strong role for cross-orientation inhibition, they do suggest that selective activation of inhibitory circuitry can profoundly alter the layout of orientation domains in the cortex, perhaps by selectively altering the balance of converging excitatory inputs to a region. Supported by EY07023.
651.1 CHARACTERIZATION OF CHLORIDE CURRENTS RECORDS FOUND IN DISASSOCIATED RAT TASTE CELLS. Xiong-Dong Sun* and M. Scott Herens, Indiana University School of Medicine, Muncie, IN 47306.

We have characterized previously unidentified chloride currents from rat taste receptor cells using circumvallate and foliate papillae dissociated with papain. We made voltage- and patch clamp technique in the whole cell configuration. Chloride currents were identified as outward currents under conditions of 159 mM (Cl-) and 34 mM (Cl-) by pharmacological isolation with 300 μM DIDS or 100 μM Niflumic acid. Currents reversed close to the expected Nernst potential and were observed in less than 5% of the whole-cell recordings. When external chloride was lowered by isethionate or gluconate substitution, currents were reduced and the reversal potential shifted accordingly. Further isolation of these currents by blocking potassium currents with a combination of TEA and 4AP provided similar results. Chloride currents were additionally recorded as inward tail currents that appeared as inwardly rectifying at membrane potentials negative to the predicted chloride reversal potential. They were sensitive to both 500 μM DIDS and to 100 μM Niflumic acid as well as lowering external chloride concentration by ion substitution. Moreover they exhibited an inactivation that had temporal and voltage dependent components to it. Supported by NIH DC00401.

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

Denatonium (DN) is a potent bitter substance. Responses of isolated mudpuppy taste receptor cells to DN were studied by imaging with fura-2 and whole-cell recording. We identified receptor cells by staining with a Cy5-apical surface antibody. In 87% of receptor cells, DN (5 mM) increased [Ca2+]i by 56.8 ± 8.8% from a resting level of 75.5 ± 2.8 mM (n = 26). The response always began in the apical tip of the receptor cell, even though the DN was applied to the entire cell. The response to DN was present in a Ca2+-free external solution, but was reduced in magnitude by approximately 75%. Niflumic acid (1 μM), a P2X4 receptor blocker, did not alter the response to DN. DN (10 μM) had no direct effect on [Ca2+]i and failed to block the DN responses. These results suggest that DN increased [Ca2+]i mainly from thapsigargin-sensitive, rydnoim-sensitive Ca2+ stores in the majority of taste cells. A small subset of DN-sensitive cells also responded to caffeine (20 mM), suggesting that caffeine-sensitive Ca2+ stores may also contribute to bitter transduction in some taste cells. Whole-cell recording showing that DN potentiated outward currents in most taste receptor cells. In some receptor cells and in all non-receptor cells, DN either blocked, or had no effect on, outward currents. Pharmacological experiments indicated that the DN-increased outward currents were carried by K+ and Cl- ions. Taken together, these data suggest that DN increases Ca2+, which subsequently deactivates K+ and Cl- currents in taste receptor cells. Supported by NIH grants DC00244 and DC01076 to SCK.

651.3 VERTEBRATE TASTE RECEPTOR CELLS HAVE NOVEL CATIONIC CHANNELS ACTIVATED BY QUININE. Takashi Tsunenari*, Yukako Hayashi*, Manabu Ohta, Takashi Kurimoto, Akimichi Khan, and Hiroshi Morik1, 1 Res. Ins. for Physiological Sciences, Myojo, Okazaki 444, Japan. 2 Dep. of Physiology, Keio Univ. Sch. of Med., Shinjuku, Tokyo 160, Japan.

Quinine is known to be a bitter substance for various vertebrates. Whole-cell recordings under voltage-clamp were made from isolated taste receptor cells of bullfrog (Rana catesbeinian) while quinine hydrochloride was applied through a pipette. Quinine stimulation elicited an inward current at the resting potential. When the blocking effect of quinine on K+ currents was eliminated by Cs+ pipette, the current–voltage relation was linear (the reversal potential: -16 mV) and showed a clear increase in membrane conductance by quinine stimulation. The quinine–activated conductance was cation selective. The relative permeability ratio was PNa/PK = 0.42 ± 0.5. The quinine–activated cationic conducting channel was suppressed by Ca2+, sharing a common feature with quinine responses in salivary glands. We therefore conclude that this novel cationic channel plays a crucial role in the bitter perception. Bitter–induced depolarization in taste cells has been explained by a blocking effect on K+ channels. It, however, seems likely that taste receptor cells have, at least, two independent pathways for the detection of a bitter substance.

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

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

651.5 ELECTROPHYSIOLOGICAL ACTIONS OF QUININE ON DISASSOCIATED RAT TASTE RECEPTOR CELLS. Yuque Chen* and M. Scott Herens, Indiana University School of Medicine, Muncie, IN 47306.

The electrophysiological actions of quinine, a classic bitter stimulus, were studied on dissociated rat taste receptor cells from foliate and circumvallate papillae using patch-clamp techniques in the whole cell recording configuration. Its most profound action was a dramatic inhibition of outward potassium currents. Inhibitions could be measured from 10^5 to 10^7 M, a concentration range that coincides well with behavioral observations in rats. At the higher range inhibition of potassium currents was both complete and reversible (97% inhibition at 10^7 M). At lower concentrations inhibition was more profound on the sustained portions of the current, leaving a transient voltage-dependent current. This may suggest a site of action on delayed rectifiers. Input membrane resistance, on the order of 10 GΩ was increased by quinine by 10 - 20%. We tested for the presence of a quinine-induced current but none could be observed, although potassium currents could also be measured. Quinine inhibited sodium current by 20 - 30% but increased calcium currents (measured with barium as a current carrier). Under whole-cell clamp conditions, some broadening of the action potential was noted in the presence of quinine. The action potential amplitude was decreased but the threshold was not changed. Supported by NIH DC00401.

651.6 RESPONSES TO GLUTAMATE IN ISOLATED RAT TASTE CELLS. A. Bigiani, B. D. Delay, N. Chaudhari, C. S. Kinnamon and S. D. Roper*, Rocky Mt. Taste & Smell Center, Denver, CO 80262, and Dept. Science and Biomedicine, Università di Modena, 41100 Modena, Italy.

Glutamate is an important taste stimulus for humans and animals alike. A metabotropic glutamate receptor, mGluR4, is selectively expressed in rat taste buds and may be responsible, in part, for transducing glutamate taste (Chaudhari, et al. 94). Yet, the mechanism of chemosensory transduction for glutamate is still unknown. We have recorded responses from single cells in isolated rat taste buds, using perforated patches. 20 mM L-glutamate was dissolved in a modified Tyrode solution (maintaining iso-osmotic solutions), and bath-applied to taste cells. Applying glutamate reduced the inward holding current at -80 mV by 5.3 ± 2.9 pA (n = 13; mean ± SD). The glutamate response decreased upon depolarization, with an apparent reversal potential of -11 ± 3 mV (n=2), close to the equilibrium potential for Cl (~15 mV). An increase in cell input resistance was observed during the glutamate response (4.7 ± 10.1 GΩ, n=1). Glutamate had no effect on the inward Na+ and outward K+ currents. Applying 0.85 mM L-AIP, a selective agonist for mGluR4, mimicked the effects of glutamate. However, at this concentration, the responses were much smaller. These data suggest that the activation of mGluR4 and the consequent modulation of a Cl conductance may be key events in the taste transduction process. Supported by NIH grant DC00374 and Ital. Ministero della Università e della Ricerca Scientifica.
651.1 CHARACTERIZATION OF BITTER RECEPTORS THAT ACTIVATE GUSTATION AND TRANSUDIN IN TASTE MEMBRANES. L. Ruiz- Avila* and B. M. Margolskee. Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, NJ 07110.

Bitter and sweet tastes are believed to be transduced through heteroregic G-proteins, which activate cell membrane receptors to intracellular effector enzymes. The taste receptors have not yet been functionally characterized nor molecularly identified. Neurons (putative primary cells) preferentially couple to the few G-protein alpha subunits; notably transudin and gustducin (which is absolutely taste specific). These two proteins are closely related at the amino acid level, and have been shown to activate specific phosphodiesterases (Ruiz-Avila et al. 1995), thereby regulating the concentration of cyclic nucleotides inside the taste cells and consequently modifying the activity of taste cell cyclic nucleotide-regulated channels (Berglund, 1993). We have reconstituted a taste responsive system using bovine or murine circumvalate papillae membrane preparations and purified bovine rod transducin or recombinant gustducin as exogenously added reporters. Both transducin and gustducin are specifically activated by receptors present in taste membranes and this activation is enhanced by the bitter compound denatonium (100 μM to 10 mM). Transducin/gustducin activation by denatonium-responsive receptors can be competitively inhibited with a peptide derived from the receptor interaction domain of transducin. The denatonium receptor-G protein interaction also requires βy subunits. Mutations in the βy-terminus of gustducin affect its interaction with rhodopsin and modulate its interaction with the denatonium responsive taste receptor. L. R-A is a Fulbright scholar from the Spanish MEC-Fulbright postdoctoral program.

651.2 MULTIVARIATE CLASSIFICATION OF RAT TASTE RECEPTOR CELLS BASED ON ULTRASTRUCTURAL MEASURES OF NUCLEAR MORPHOLOGY. D. W. Pampiglione, C. Yu, A. Z. Murphy* and D. V. Smith. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1599.

Mammalian taste receptor cells are organized within taste buds on the tongue and other oral epithelia. These cells have been classified into light, dark, and intermediate cells on the basis of ultrastructural criteria. Light cells are said to have large, round or ovoid, smooth nuclei with little heterochromatin and a relatively electron-dense cytoplasm. Dark cells are characterized as having elongated, invaginated, heterochromatic nuclei and electron-dense cytoplasm. Intermediate cells lie between these extremes. Both the occurrence of large numbers of intermediate cells and the dependence of the light/dark categorization on the parameters of fixation suggest that such a classification may be somewhat arbitrary. Ratlets were perfused with 1% paraformaldehyde/5% glutaraldehyde in 0.1 M sodium cacodylate buffer, tongues were removed, and semi-sliced thin sections were cut transverse to the longitudinal axis of the vallate taste buds. To develop objective criteria for cell classification, the nuclear morphology of 95 cells was quantified with several measures that are purported to correlate with the light/dark cell classification (nuclear area, eccentricity, irregularity, and heterochromatin). These measures discriminated well among taste cells. The resulting matrix of measurements was analyzed with both hierarchical cluster analysis (SPSS CLUSIS) and multidimensional scaling (SPSS ALSCAL). The multivariate distributions of the cell nuclei were continuous, suggesting that the light/dark cell dichotomy may represent points along a continuum of morphological types. Supported by NIH grant DC00347 to DVS.


We are recording responses of single units in the chorda tympani (CT) nerve of hamsters (Mesocricetus auratus). Stimuli include sodium salts, sweeteners, the saccharin-sodium chloride mixture, sucrose-Polyose, and the binary mixture of sucrose plus quinine.HCl (Qu). We have determined the total number of spikes during the initial 5 s of response minus the average spontaneous rate for the unit. Preliminary results show that all tested 0.1 M sodium salts are effective for NaCl-best units. They show an even more striking response to NaCl. Furthermore, their responses are amplified by amiloride than the taste of Na-gluconate, which is consistent with the effects of amiloride in the rat. However, amiloride affects only the sour side taste of these salts and produces no effect on their saltiness. This suggests a correlation between stimulation of the apical ion channels and the sour taste of Na. These apical ion channels have been shown to be more permeable to Li+ than to Na+. LiCl also has a larger sour component than NaCl. Therefore, we hypothesized that the taste of LiCl would be more greatly suppressed by amiloride than the taste of NaCl. Human subjects estimated the taste intensity and qualities of several concentrations of NaCl, LiCl, and KCl. Amiloride suppressed the sourness of LiCl more than that of NaCl, there was no effect on the taste of KCl. Estimates of the saltiness of these stimuli were unaffected by amiloride treatment. We propose that Na+ and Li+ stimulation of amiloride-sensitive ion channels elicits a sour taste; saltiness may arise via the paracellular pathway.

Supported by NIH grant DC00347 to DVS.

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

Previous work from our lab has shown that dietary sodium restriction instituted early in prenatal development produces anatomical and functional changes in the developing gustatory system. In particular, neurophychological recordings from the brainstem in newly weaned rats have revealed a rearrangement of the CT terminal fields within the nucleus of the solitary tract (NTS) in restricted rats, while gustatory evoked potentials altered in restricted rats compare to control rats. To determine whether the NTS recordings of restricted rats replete with sodium show hyper-responsive activity to only sodium salts, which technique could indicate additional dendritic alterations of the presynaptic neurons from the NTS, we examined the neurophysiological to evaluate the developmental time course of the alterations seen in the NTS in response to the NaCl restriction paradigm, the chorda tympani nerve was labelled in a developmental series with a 35K biotinylated dextran amine neuronal tracer. Ganglion cells in sodium restricted and control rats. Results show that the alterations begin at early postnatal time points, and that these alterations continue until adulthood. To determine whether the developmental sodium restriction could alter the pathway which the afferent fibers follow to their target. Supported by the NIDR training grant (B.R.W.) and NIH DC 00407.


We have recently shown that a neuron’s most effective stimulus (“best response”) is a relatively poor predictor of neuronal morphology. The present investigation was conducted to test the hypothesis that individual “best response” groups may be comprised of physiologically-distinct neuronal subtypes that do exhibit unique morphologies. Glass micropipettes filled with horseradish peroxidase were used to test the response of NTS neurons to 0.1M NaCl (N), 0.01M HCl (H), 0.01M quinine (Q) and 1.0 M sucrose (S). Seventy-one neurons that responded to one or more tastants were injected with horseradish peroxidase. The peroxidase filled neurons were used to perform a cluster analysis (SPSS) and the results of the cluster analysis were used to define physiological subtypes. Analyses of variance were then performed to investigate relationships between neuronal morphology and physiological response properties. We were able to identify a number of trends that appear to deserve further examination with a larger data set. There was a trend (F=2.2, df=4/61; p=0.08) between mediodistal extent and response to a subset of the neurons that responded best to NaCl (N+H=Q or S) more widespread than the N-best or Q-best neurons. This N-best subset was also more broadly tuned than other subsets (F=4.2, df=4/61; p=0.03) except the Q-best neurons and there was a trend (F=1.9, df=4/61; p=0.12) that indicated this group had a larger soma-dendritic receptive field (in the coronal plane) than other groups. These data indicate that physiologically-defined subsets of the traditional N-, H-, Q- and S- 'best' categories may provide important insights into structure-function relationships in the NTS. Supported by DC01674.
651.13 MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF ACUTELY ISOLATED NEURONS FROM THE GUSTATORY NUCLEUS OF THE SOLITARY TRACT. R.M. Rieder* and D.Lu. Dept. Biologi and Materials Sciences, Sch. of Dentistry, Univ. of Michigan, Ann Arbor, MI 48104-1078.

To study the functional properties of identified neurons in the rostral (gustatory) nucleus of the solitary tract (NST), we have acutely isolated the cells so that direct comparisons can be made between structural and biophysical properties of the neurons. Neurons were isolated from 300 µm thick horizontal brain slices from rats aged 7-20 days. The NST was dissected from the slices, placed in HEPES buffer containing 0.5% protease type 23 and then triturated with a series of different diameter, fire-polished Pasteur pipettes. Neurons were then positioned on a poly-L-lysine coated coverslip in a plastic petri dish and superfused with oxygenated saline. Viewed with an inverted microscope, elongate, multipolar and ovoid neurons could easily be distinguished. These same neuron morphologies have been described in NST by us and other investigators using a variety of morphological techniques. We recorded from the neurons using the whole cell configuration of the patch clamp technique. The isolated neurons had mean resting membrane potentials of -69 ± 14 mV, a mean input resistance of 308 ± 66 MΩ and overshooting action potentials. When depolarized the neurons either fired a regular train of action potentials or a short burst of action potentials that then telemarinated. For some neurons the initiation of the regular train of action potentials was delayed by a brief membrane hyperpolarization. These intrinsic properties are similar to those we have previously reported in whole cell recordings from NST neurons in brain slices (J.Neurophyiol. 67:1659, 1992). This study shows that neurons acutely isolated from the NST maintain both their morphological and biophysical properties.

Supported by NIDCD grant DC00288 to R.M.B.


We have recently established that there are eight morphologically-defined gustatory cells types in the NST. Cells in one of these cell types have small somata (<125 µm²) and a high density of dendritic swellings (>0.5µm). To investigate the ultrastructural characteristics of terminals that contact the dendritic swellings, cells of this type were processed for electron microscopy. These cells were tested for their responses to an array of tastants, filled with Neurobiotin, reacted with DAB and flat embedded in resin. We were able to verify that dendritic swellings receive synapses. These terminals are of the "primary-like", "sp" and "mp" (small and medium pleomorphic) types as defined by Whitehead (JCN, 332, 1993). We have classified the responses of these cells. A disproportionate number of them are NH4CI-best and HCI-best. One cell of this type only responded to two (HCl and NH4Cl) of the eight tastants and was HCl-best. This cell receives terminals onto dendritic swellings and the terminals contacting the swellings have rounded or pleomorphic vesicles. We have verified that synapses of all morphological types defined by Whitehead are found in the area in which our gustatory cells are labelled, yet several synaptic types do not appear to contact dendritic swellings.

Supported by DC01074.

651.15 FOREBRAIN PROJECTIONS TO THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT IN THE HAMSTER. M.C. Whitehead*, A. Bergula and K. Holliday, UCSD, La Jolla, CA 92039.

The nucleus of the solitary tract (NST) is the first central site of taste information processing. Specific subdivisions of the NST receive taste afferent input and contain interneurons and projection neurons that engage ascending or premotor taste pathways. The forebrain projects to NST and can influence taste responses, but the anatomical relationship between forebrain inputs, primary inputs and NST cell types is not understood. To evaluate this question we have examined the projection to NST of the central forebrain subdivision containing gustatory cortex. Injection of BrdU into gustatory cortex, the site of most forebrain-NST cells, labeled axon endings confined to the rostral NST. These endings were concentrated in the caudal central and ventral subdivisions. In the rostral central subdivision cortical endings are positioned influence interneurons that include taste afferent synapses, synapses of presumed inhibitory interneurons, and neurons that project to the parabrachial nucleus. In the ventral subdivision cortical endings synapse among premotor neurons that ultimately influence salivatory and oromotor outflow.

Supported by NIH grant DC02045.

651.16 EXPRESSION OF c-FOS IN THE RAT PARABRAChIAL NUCLEUS FOLLOWING INJECTION OF MONOSODIUM GLUTAMATE AND OTHER TASTANT STIMULi. S. M. Roper*, J. C. Kimamati and S. B. Roper*. Dept. of Biological Sciences, Univ. of Denver, Denver, CO 80209 and Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

In rodents, the pontine parabrachial nucleus (PBN) includes the second order gustatory relay in the CNS. In order to compare the localization of c-Fos responses to monosodium glutamate (MSG) with that of responses to other taste stimuli, we used a monoclonal antibody to detect Fos protein expression in PBN neurons following ingestion of tastants. Adult rats were water-deprived and trained to drink during a one-hour period each day. On the experimental day, rats were given either distilled water or a tastant solution during the drinking period. Brains were subsequently fixed by perfusion and the pontine regions sectioned at 50m in a Vibratome. Fos-like immunoreactivity (Fos-ir) was visualized using a rabbit polyclonal antibody (Santa Cruz Biotechnology, 1:5000). Although sparse Fos-ir appeared throughout the PBN, the location of the greatest concentration of Fos-ir cells occurred in different PBN subnucleus, depending upon the tastant solution ingested. In animals that received distilled water, a distinct cluster of Fos-ir neurons appeared in the dorsal subnucleus (dls) of the lateral PBN. Fos-ir was similarly localized following ingestion of 500m MSG. Rats that were given 3mM quinine HCI showed the greatest Fos-ir in the ventral region of the external lateral subnucleus (els) and the external medial subnucleus (ems). In animals that received 500m MSG plus 30um amiloride, Fos-ir was concentrated in the els in an area more dorsal than that observed in animals receiving quinine HCI. The region of greatest Fos-ir in response to 500m MSG and Fos-ir corresponds to the zone in which Fos-ir has been observed following intraperforato injection of LCl (Yamamoto et al., Neuroreport 3:1049, 1992).

Supported by NIH DC01553, NIH DC02044 and NIH DC00374.

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

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

Supported by NIH grants DC01263 to MBV and DC00347 to DVS.
DOPAMINE INHIBITION OF NUCLEUS ACCUMBENS CELL ACTIVITY OCCURS VIA TWO INDEPENDENT MECHANISMS: EVIDENCE FROM IN VITRO AND IN VIVO INTRACELLULAR RECORDINGS. A.A. Glivec and P. O'Donnell, Dept. Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

The excitatory or inhibitory nature of dopamine (DA) receptor activation has been a topic of controversy for years. This study analyzes the effects of DA-related drugs on indices of cell excitability in vivo, and on the pattern of spontaneous firing observed in vivo. Forty-five rats were isolated from litters beginning at 3 days and maintained under these conditions. Perfusion with the D1/D2 agonist apomorphine (10-150 μM) caused the membrane to depolarize from -48.8 ± 8.3 mV to -57.3 ± 10.5 mV without concurrent changes in input resistance or the firing rate. The amplitude of current injection required to trigger spike firing was increased from 0.28 ± 0.18 nA to 0.49 ± 0.32 nA (p<0.005) following apomorphine, and the membrane potential at which the cells fired was shifted from -44.2 ± 2.6 mV to -36.6 ± 11.1 mV (p<0.001). This decrease in excitability was also obtained with the combined perfusion with a D1 agonist ( SKF89395; 1 μM) and a D2 agonist (quinpirole, 10 μM), but not by administration of drug alone.

Intracellular recordings were made in vivo from 2 acumbens cells that exhibited a bistable membrane potential. Systematic administration of a combination of SKF89395 (10 mg/kg, i.p.) and quinpirole (10 mg/kg, i.v.) reduced the probability of the membrane switched to the depolarized, active state from 1/2 to 0.55 in both cells tested. Since w-1ave previously shown that responses to prefrontal cortical inputs can be elicited only during the desensitized state (J.Neurosci,1995;15:326-349), a decrease in the frequency of depolarizing periods would result in a functional blockade of cortical inputs in the nucleus accumbens. Furthermore, both in vivo and in vitro actions appear to be mediated by excitation of D1 and D2 receptors. Thus, the DA-mediated depolarization of acumbens cell membranes, the combined effects of a decrease in cell excitability and the reduction in transitions to the depolarized state would independently produce an inhibitory influence on nucleus accumbens activity. Supported by MH42277, 45156, 0105S (AAG), and Tourette’s Syndrome Association (P.O.D.).


Excitotoxic lesions of nucleus accumbens septi (NAS) were previously shown to have a delayed effect on the intracranial self-stimulation (ICSS) response in rats. The present study addressed the ability of kainic acid-induced lesions of NAS to affect acquisition of ICSS. Mice with bilateral lesions of NAS or of the ventral tegmental area and bilateral cannules were implanted into the NAS. Seven days postoperatively two groups of animals - lesioned and controls - were trained by repeated food reward for contingent electrical stimulation (continuous reinforcement schedule). Fourteen 25-min training sessions were performed once a day by an experimental procedure in the group of animals, for the group of animals responding for electrical stimulation by the end of the training period were characterized after histological verification of cannulae electrode placement and evaluation of the size of lesions. The kainic acid-induced damage was generally restricted to the NAS and minor damage to caudatus nucleus. Only 1 out of 10 kainic acid-lesioned animals responded more than 200 times in a session (mean of 45), whereas 5 out 6 control sham-lesioned successfully acquired the SS response (p=0.05, chi-square test). These data suggest that NAS is necessary for the acquisition of ICSS. (Supported in part by grant NWO 07-13-087 of Dutch Medical Council).

CORTICO-STRIAL SUBSTRATES OF PREPULSE INHIBITION OF ACOUSTIC STARTLE IN THE RAT. J.L. Wiant, M. H. Kudo, S. B. Caine and N. R. Eganro, Deps. of Neuroscience and Psychiatry, University of California San Diego, La Jolla, CA 92037-0804.

Inhibition of the acoustic startle reflex by a weak "prepulse" is an operational measure that can be studied in both humans and rats. Since deficits in prepulse inhibition (PPI) are characteristic of several neuropsychiatric disorders including schizophrenia, it is of interest to examine the neural substrates underlying PPI. Importantly, recent studies using neuroimaging and neuropsychological methods have identified abnormalities within limbic-cortical circuits in schizophrenia patients. In the present experiment, we examine limbic cortico-striatal substrates of PPI in the rat. Preliminary data shows that PPI is disrupted after infusion of the non-NMDA agonist AMPA into the nucleus accumbens core and shell subregions. This effect of AMPA in the core can be blocked by systemic pretreatment with the D2 antagonist haloperidol (0.1mg/kg), whereas that of AMPA in the shell appears not to be blocked by haloperidol. Infusion of NMDA (8μg) into the ventral subiculum of the hippocampus significantly reduces PPI. This effect of NMDA on PPI is not prevented by the non-NMDA antagonist CNQX (25μg) infusion into the NAS core or shell subregions, or by systemic injection of haloperidol. PPI is reduced after infusion of either NMDA or the NMDA antagonist AP5 into the basolateral amygdala, but not after quinpirole (0.15mg/0.3μl) lesions of the basolateral amygdala, indicating that the NMDA receptors are required for the effect of NMDA on PPI. Further, we are now studying the potential contribution of the prefrontal cortex and its connection with the NAS to the modulation of PPI. Our results suggest that glutamatergic limbic-cortical substrates play an important role in the regulation of sensorimotor gating in the rat.


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

DOPAMINE RECEPTOR MODULATION OF WHOLE CELL SODIUM CURRENT IN ACXUALLY DISOCIATED RAT NUCLEUS ACCUMBENS NEURONS. S. Jiang and J. S. Frankel, Miami Dept. of Neuroscience, FINS, The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064-3095.

The nucleus accumbens (NAc) is a ventral striatal region that serves as an interface between limbic and motor systems and thereby plays essential roles in motivational aspects of behavior. Therefore, NAc neurons and their dopaminergic (DA) innervation have become the subject of considerable interest as they are currently being studied under a variety of conditions. In the present study, we used intracellular current clamp and whole-cell voltage clamp techniques to investigate dopamine receptor-mediated modulation of TTX-sensitive sodium currents in NAc neurons. In adult rat NAc slices, both application of DA (25 μM, n=7) significantly hyperpolarized NAc neurons, increased the amount of intracellular current injection required to generate action potentials, increased the spike threshold and decreased spike amplitudes. Similar results were observed in two neurons with application of the D1 receptor agonist SKF 38393. For whole-cell recordings, NAc neurons from 1-4 weeks old rats were acutely dissociated. Only medium-sized neurons (6-12 μm) were used. Application of DA (10-25 μM) reduced peak INa (I50), SKF38393 (1-3 μM) also reduced peak INa (10/12), an effect that was completely blocked by the DA D1 receptor antagonist SCH 23390 (5 μM). The degree of inhibition produced by SKF38393 was 6-50% of control. The effect reduced peak INa, without affecting the activation pattern. Instead, the steady-state inactivation curve was shifted about 5 mV in the negative direction. The DA D1 receptor antagonist SCH 23390 (5 μM) exerted diverse effects on INa in that two of 8 neurons responded with increased current, whereas four responded with decreased INa. Both of the effects were completely blocked by DA D1 receptor antagonist-stimulated cAMP (10 -6 M). D1 receptor activation reduction INa, results in hyperpolarization of NAc neurons and decreased excitability. The effects of DA D1 receptor activation appear more complex and variable, perhaps mediating multiple receptor involvement. (Supported by DA04693 and DA00207 to F. W.)
**652.1** MODULATION OF SUBSTANTIA NIGRA DOPAMINE NEURON ACTIVITY BY MUSCARINIC ANTAGONIST ADMINISTRATION. C.L. Todd, W. Cameron*, and A.A. Grace. Dept. of Neuroscience, Dept. of Psychiatry, Center for Neuroscience, University of Pittsburgh, PA 15260.

Benztropine (Ben) is a muscarinic antagonist that is used clinically to treat acute dystonia, an extrapyramidal side effect induced by antipsychotic drugs (APDs). Acute dystonia typically occurs within the first four days of treatment with APDs, and is more common when high-potency APDs (e.g., haloperidol) are used. Although the effects of APDs on the firing rate of substantia nigra (SN) DA cells have been well characterized, little data regarding the effects of Ben on these cells has been reported. Therefore, in the present study we examined the effects of Ben on the DA cell firing of rats in vivo. Each experiment included a control period with no drug, followed by the injection of Ben (1 mg/kg, i.p.) and an additional 30 min. In one group of animals, the effects of Ben on the mean firing rate of DA cells were measured. Ben administration increased the mean firing rate of DA cells in a dose-dependent manner. In another group, the effects of Ben on the mean firing rate of DA cells were measured in vivo. Each experiment included a control period with no drug, followed by the injection of Ben (1 mg/kg, i.p.) and an additional 30 min. In one group of animals, the effects of Ben on the mean firing rate of DA cells were measured. Ben administration increased the mean firing rate of DA cells in a dose-dependent manner.

**652.2** HIPPOCAMPAL ACTIVATION SUPPRESSES VTA DOPAMINE CELL FIRING. A POTENTIAL ROLE FOR HIPPOCAMPAL REGULATION OF PHASED DA RELEASE. L.G. Hardten* and A.A. Grace. Dep. of Neuroscience and Psychiatry, UCSD, San Diego, CA 92103.

Post-mortem studies of the brains of schizophrenics suggest that hippocampal damage may play an important role in the etiology of this disorder. Although several studies have examined the impact of hippocampal lesions on dopamine (DA) function in striatal targets, the influence of hippocampal activity on DA cell firing is not yet clear. In the present study, we examined the effects of hippocampal activation on VTA DA cells recorded in rats. In one set of experiments, electrical stimulation consisting of single pulses with a duration of 0.2 ms and intensity ranging from 0.5-1.0 mA were applied to the fornix. Activation of the fornix potentiated 27/31 (87.6%) of VTA DA cells tested. The mean onset latency of the inhibition was 33.8 ± 4.2 ms (-1.9 ± 0.0 ms) and the mean duration of inhibition was 19.8 ± 2.3 ms (48.0 ms). To address the possibility that the observed suppression was due to inadvertent activation of non-hippocampal projections, direct stimulation of the CA/subicular region was performed in a second set of experiments. Similar results were obtained, direct hippocampal activation resulted in inhibition of VTA DA cell firing in 33/47 (70.2%) of the neurons examined. The mean onset latency of the response was 44.7 ± 6.5 ms (10.9 ± 5.5 ms) and the mean duration was 127.8 ± 40.6 ms (47.5-100 ms). Because there are no direct projections from the hippocampus to the DA cells of the VTA, the observed inhibition is likely to be mediated by polysynaptic pathways involving the nucleus accumbens or prefrontal cortex or both.

The finding that hippocampal activation suppresses DA cell firing, suggests that hippocampal damage reported to be present in schizophrenia could potentially disrupt subcortical DA systems by reducing an inhibitory influence at the level of the cell body. This work was supported by NS19608. MH42217. MH10055.

**653.1** ACTIONS OF SEROTONIN ON SUBSTANTIA NIGRA PARAS RETICULATA NEURONS IN VITRO. I.M. Stanford and M.C. Lacey. Dept. of Pharmacology, University of Birmingham, B15 2TT, U.K.

The GABA containing neurons of the substantia nigra pars reticulata (SNr) constitute a major relay of basal ganglia output projecting to the thalamus, superior colliculus and the basal ganglia. The SNr has long been a target for clinical research and more recently, for voluntary movement and has been implicated in the generation of seizure activity. SNr neurons receive inputs from GABA-containing pallidal and striatal neurons, glutamate-containing mesencephalic neurons as well as afferent input from the retina. Whole-cell patch clamp recordings were made from SNr neurons from 300-400 mm paraganglionic slices of midbrain from rats 9-12 days of age. Individual neurons were visualized and recordings were made using a Nomarski optical system (Zeiss Axioskop, 80X water immersion objective). SNr neurons were easily identified as they fired spontaneous action potentials at a fast rate (range 5-8Hz, mean 15.6 ± 2.7 Hz, n = 38), showing no evidence of LI and were insensitive to 10(-5)M and met-enkephalin (10(-6)M), pharmacologically distinguishing them from dopamine cells and interneurons of the pars compacta. Serotonin (10(-8)M) caused an inward current accompaniment during fast membrane conductance changes (55-60%). The inward current was blocked by tetrodotoxin or depolarization was insensitive to 10(-7)M Cruzatropin a serotonergic microelectrode recording from SNr neurons in 400 mm thick paraganglionic slices of rat midbrain, maintained at 33-34°C and superfused with a standard medium. SNr dopamine neurons were recognized by the presence of a fast inward rectification of 5-6 mV. In a large current on hyperpolarization under voltage clamp. Synaptic potentials were evoked by stimulating a region 200-500 mm rostral to the substantia nigra using a high frequency, standard stimulating electrode, the amplitude of the response potential at between -70 and -80 mV with constant hyperpolarization current (10-30 pA).

In the presence of picrotoxin (500 nM) to block GABAA receptors, the residual synaptic potentials (EPSPs) were blocked by CNQX and APV, indicating that they are glutamatergic mediated. The broad spectrum metabotropic glutamate receptor (mGluR) agonist 1-aminocyclopentane-1,3,5-dicarboxylate (15,3R-ACPD 30 µM) caused a reversible depression of 38 ± 4% of this glutamate mediated EPSP (6/6 cells). A reversible membrane depolarization of 3-5 mV was also observed. The depression of the EPSP and the depolarization caused by ACPD was reversed by the mGluR antagonist a-methyl-DOPA (1000 nM) in 4/4 cells. These data suggest that excitatory afferents to dopamine cells possess mGluR receptors that act to reduce glutamate release from the terminals. MAW is a Wellcome Prize Student.
653.5

**ELECTROPHYSIOLOGY OF ANTISENSE KNOCKOUT OF D2 AND D3 Dopamine RECEPTORS IN NeOSTRIATAL Dopamine NEURONS.** B-C. Sun*, T. Tepper, and L.M. Tepper. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

We have shown that infusion into substantia nigra of a 19-mer antismere oligodeoxynucleotide (ODN) to 2-b of the mRNA for the dopamine (DA) D2 receptor for 6 days greatly ameliorates amphetamine-induced inhibition of firing of nigral DA neurons (Sci. Ncnemat. Abs. 20:908, 1994).

Very similar effects of antisense D2y ADN AS to D3 receptors in the inhibition of DA neurons in response to DA agonists. These effects of antisense administration to those after 6 days, suggesting a relatively rapid rate of DA receptor turnover and transport. Only D3y ADN knockout increased spontaneous firing rates (F=5.1, d=0.05, p<0.01). Control (Gh): S.3±2.3 spikes/min; D2y ADN AS: 4.3±2.4; D3y ADN AS: 3.3±1.3; D2y+D3y AS: 7.3±2.4.

653.7

**INWARD AND OUTER RECTIFICATION IN NEOSTRIATAL NEURONS DURING THE EARLY POSTNATAL PERIOD.** T. Kurie, T. Tran*, and L.M. Tepper. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Previously, we have shown that about 1/3 of rat striatal medium spiny neurons in vivo exhibit a slowly developing outward rectification (OR) at strongly hyperpolarized membrane potentials and a fast outward rectification in the deactivating direction during the early postnatal weeks (Tepper et al., 1995, 1993) with the remainder exhibiting relatively linear I-V curves. In contrast, medium spiny neurons are not inhibited by a fast inward rectification.

400-µm neuronal slices from Sprague-Dawley rats (3-11 days of age) were cut in a modified horizontal plate. Intracellular recordings were obtained from submerged slices at 32°C with glass micropipettes filled with 3 M KCl and 3.89% agarose containing 60-100 MΩ resistance.

Hyperpolarizing current pulses (150-300 ms) revealed a modest OR that became very pronounced at potentials more negative to -110 mV, resulting in a hyperpolarizing ramp. After application of 2 mM Ca²⁺ the activation range for the ramp potential and the OR shifted in the positive direction and could be inhibited at potentials more negative than -80 mV. 10 mM TEA or 300 µM Ba²⁺ (with or without Ca²⁺) blocked the ramp potential and the OR and linearized the neuron's response to hyperpolarizing current pulses. These data also increased input resistance approximately by 10% compared with the value measured at the end of the most hyperpolarized potentials in the absence of Ca²⁺. In some neurons 2-10 mM TRA revealed a Ca²⁺ sensitive time-dependent inward rectification. The outward rectification in the deactivating direction could be blocked by 3 mM, but not 300 µM Ca²⁺.

653.9

**STIMULATION OF STRIATAL D1 AND D2 RECEPTORS IS NECESSARY BUT NOT SUFFICIENT FOR SYNERGISTIC EFFECTS OF SYSTEMICALLY ADMINISTERED Dopamine AGONISTS ON SUBSTANTIA NIGRA PARSECLITICULAR NEURONS.** M. J. Twenty*, D. A. Berenson, and L. M. Tepper. ETS Neuromodulation and Neurotransmission, New York, New York 10025.

Concurrent intravenous (i.v.) administration of the dopamine D1 agonist SKF 38393 and the D2/D3 agonist quinpirole inhibits the activity of dopaminergic substantia nigra pars reticulata neurons in rats with 6-hydroxydopamine (6-OHDA)-induced lesions of midbrain dopamine cell bodies. In order to test whether striatal D1 and D2 receptor agonists were administered locally in striatum while the activity of individual SNpr neurons was recorded extracellularly in gallamine-perfused, spontaneously breathing rats. Striatal infusion of either SKF 38393 (17 nmoles, n=4), quinpirole (19 nmoles, n=9), or the combination of both agonists (45 nmoles total = 17 nmoles each) decreased the firing rate. However, the neural injection of quinpirole and i.v. SKF 38393 (3.4 mg/kg) decreased SNpr neuronal activity to 49 ± 10% (n=10) of baseline.

653.10

**SIMULATION OF THE INTRINSIC MEMBRANE PROPERTIES OF GUINEA-PIG AND RAT MEDIUM DOPAMINE NEURON INCREASES THE GABAergic RESPONSES TO DIURNAL PULSATION OF INHIBITORY INTERNEURONS.** W.-J. Yang (SPON: The Hong Kong Society of Neurosciences), Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong.

A biophysical model capable of exhibiting some of the characteristic properties of midbrain dopamine neurons in rats with 6-OHDA lesions was developed. In this model, the inhibitory interneurons of the substantia nigra pars reticulata are modeled as voltage-clamped leak channels with 3-state kinetic model of the gKCa. Calcium influx through the high-threshold calcium channel activates the gKCa. The time course of the AHP is determined by the kinetics of the gKCa rather than the intracellular calcium concentration.
HISTAMINE H₂-RECEPTOR ACTIVATION INHIBITS K⁺-EVOLED GABA RELEASE IN RAT SUBSTANIA NIGRA PARS RETICULATA SLICES

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The substantia nigra pars reticulata (SNr) plays an important role in the regulation of basal ganglia motor functions. The spontaneous activity of SNr neurones is controlled by striatal GABAergic projections and by dopamine (DA) released from terminals located in the substantia nigra pars compacta (SNC). There is evidence that histamine is also involved in the control of locomotor activity and high levels of H₂-receptors have been found in SNr (Bodine-Fowler et al., Neurosci. 52:160-189, 1993). Therefore, we set out to study whether H₂-receptor activation modulates GABA or dopamine release in SNr.

Coronal slices (300 μm thick) from SNr were labelled in Krebs-Henseleit (K-H) buffer with either [3H]-GABA (glial uptake prevented by 10 μM β-alanine) or [3H]-DA. After washing out the excess of radioactivity, slices (6-8 per chamber) were perfused with KH buffer (1 ml min⁻¹) and perfusates (collected every 4 min) were analysed by liquid scintillation spectrometry. High K⁺ (15 mM)-induced depolarisation resulted in increased (3H)-GABA efflux (2.8 ± 0.2 fold of basal release) and (3H)-DA efflux (1.8 ± 0.0 fold of basal), as estimated at the peak of tritium release. The selective H₂ agonist imipenem (1 μM) decreased (by 53 ± 8%) K⁺-evoked (3H)-GABA release. This effect was fully reversed by theophylline (1 μM), a selective H₃ antagonist. Depolarisation-induced (3H)-DA release was slightly reduced by imipenem, but the inhibition failed to yield statistical significance. These results suggest that histamine may regulate SNr function by inhibiting GABA release via H₂ receptors mainly located on striatonigral terminals.

MUSCLE: CELLULAR AND MOLECULAR PHYSIOLOGY

ALTERED MYOSIN mRNA AND PROTEIN CONTENT IN RAT SOLEUS AND TIBIALIS ANTERIOR MUSCLE FOLLOWING FOREIGN REINNERRVATION.

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Reinnervation by foreign motor units may strongly influence myosin heavy chain (MHC) expression. MHC content may be controlled by both gene transcription and translation, thus MHC mRNA and protein were examined in the tibialis anterior (TA) and soleus (Sol) following denervation and reinnervation. The sciatic nerve of adult rats was transected and repaired by either epineural sutures or a silicon tube (10 group). Following 8 or 32 wks of recovery, the muscles were excised, weighed, and frozen. MHC protein content was quantified by SDS-PAGE and mRNA levels with the RNase Protection Assay which allows comparison of relative levels of the 4 adult MHCs within the same sample. Control Sol contains predominately type I MHC; however, 8 wks following nerve injury, there were increases in fast and slow MHC mRNA within the different classes of the proteins. However, by 32 wks, increases in both fast and slow MHC mRNA and protein, especially slow, were found with concomitant decreases in slow MHC. The relative amounts (% total) of fast MHC mRNA and protein increased from 5% in control Sol to ~45% at 32 wks with tube repair. Similarly, 32 wks after tube repair in the TA, fast MHC mRNA and protein increased primarily at the expense of type I MHC. In control TA, the mRNA content was ~77% while 32 wks following tube repair, the mRNA ranged from 4 to 50%. These findings emphasize the role that motorneurons play in regulating MHC expression. The timing of these changes indicate that isoform switching can be detected at the mRNA level considerably before detection of the new protein. This work was supported by the Research Service of the VA.

MYOSIN HEAVY CHAIN COMPOSITION OF SOLEUS MUSCLES IN SPINAL CORD INJURED AND CONTROL HUMANS.

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

MHC PROTEIN AND mRNA EXPRESSION IN RAT SOLEUS MOTOR UNITS AFTER SCIATIC NERVE INJURY.

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Although the nerve has been shown to affect the myosin heavy chain (MHC) isoform expressed in a muscle, little is known about the expression of the MHC mRNA transcripts. We chose to examine the influence of the nerve by studying MHC protein and mRNA expression in motor units of the rat soleus Sol). Sciatic nerves of adult female rats were transected followed by epineural suture repair. After a period of 16 weeks, motor units from the Sol were functionally isolated, physiologically tested, and glycoendepleted. Motor unit fibers were identified in serial cross-sections that had been labelled with MHC antibodies and mRNA probes. Previously, we demonstrated that only 10% of the neurons reinnervating the Sol were from the original motor pool and that the Sol was reinnervated by twice as many motorneurons (Bodine-Fowler et al., Neurosci. Abstr. 20: 256, 1994). Whole muscle tetanic force was reduced from normal, as was the average fiber size. Motor units (n=6; 2 slow, 2 fast, and 2 small) ranged in force production from ~15 to 180 mN, in contrast to the 28 to 150mN range in control motor units. Contraction time ranged from 12 to 30 mS, as opposed to 25 to 88 mS in control motor units. Only 3 of the 6 motor units were fatigue resistant. Type 2x MHC, normally found not in rat Sol, was abundant in all muscles tested. The fibers in each of the motor units were composed of one type of MHC. Interestingly, each of the visualized units expressed only one mRNA transcript, though a large number of non-motor unit fibers contained two or more transcripts. These results indicate that after nerve injury motor unit fibers have acquired a similar degree of homogeneity with respect to protein and mRNA expression. This work was supported by the Research Service of the VA.

RHABDOMYOSARCOMA CELLS TRANSFECTED WITH CONNEXIN-43 SHOW AN INCREASE IN MYOGENIC DIFFERENTIATION.

Department of Anatomy, Univ. of Western Ontario, London, Canada. Beijing Institute for Cancer Research, Beijing, China.

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

This work has been supported by the Muscular Dystrophy Association of Canada.
**MUSCLE: CELLULAR AND MOLECULAR PHYSIOLOGY**

**654.5**

**EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR AND ITS RECEPTORS IN THE HUMAN FETAL MUSCLE.**


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

(Supported by Telethon-Italy to F.G.).

**654.6**

**TISSUE-ASSOCIATED COUNTERPARTS OF α2-MACROGLOBULIN IN MOUSE SKELETAL MUSCLE.**


Proteinase inhibitors normally regulate proteolysis in numerous tissues and cells. However relatively few data are available in literature about protease inhibitors in normal and diseased muscle. The present experiments deals with the immunohistochemical and immunocytochemical detection of α2-macroglobulin (α2M) and inter-α-trypsin inhibitor (ITI), two major protease inhibitors in mouse skeletal muscle. Immunoperoxidase histochemistry (ABC method) was done on cryostat sections and finally immunocytochemical staining with a peroxidase-conjugated anti-(α2M) showed immunoreactivity occurred widespread in extracellular structures (peri-endomysium, blood vessel wall) as well as inside a half of the muscle fibers.

**654.7**

**IMMUNOSTAINING OF ISOLATED ADULT RAT SKELETAL MUSCLE FIBERS WITH ANTI-α2 M PUMP ANTIBODIES.**


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

**654.8**

**ION-DEPENDENT ALTERATIONS IN METABOLIC GENE EXPRESSION IN SKELETAL MUSCLE.**

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

(Funded in part by grants from the Veterans Administration and the National Institutes of Health.)

**654.9**

**EQUATORIAL MORPHOLOGY OF SLOW AND FAST INTRAFUSAL FIBERS IN CHICKEN LEG MUSCLE SPINDLES.**

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Equatorial regions of chicken leg muscle spindles were examined to review the notion that at midgapper their intrafusal fibers present a uniform structure, regardless of fiber type. Based on myosin heavy chain composition, intramuscular fibers were either classified as slow (S and M fibers) or fast (U fibers) (Maier and Zal, Am. J. Anat. 187:338-346, 1989). Staining for α-bungarotoxin was exceedingly rare on both fiber types, indicating that neither fiber type received a significant motor innervation at the sensory region. Immunostaining for acetylcholinesterase, on the other hand, was present as a continuous or occasionally interrupted band. The equator in either fiber group, but only on the non-synaptic side, away from myosynaptic junctions. Sensory terminals making contact with fast U fibers were frequently claw-shaped, while those connecting to the slow M and S fibers were typically less curved. The periferal sheath was shortest in M fibers, and on average, there were fewer myosynaptic contacts on slow than on fast fibers. In sections which were immunostained for filamentous actin by the phalloidin toxan reaction, at the point where the periferal sheath was most prominent, actin was restricted to a narrow crescent on the synaptical side. This paucity of actin extended along the equator for a greater distance in slow than in fast fibers, denoting a more extensive distribution of slow MHC. As seen in cross sections, slow MHC was concentrated on the periphery of fibers. There are then at the equator of chicken intramuscular fibers some fiber type-specific structural elements, but they are much more pronounced than in mammalian spindles. Physiological experiments are required to determine if these morphological differences relate to unique mechanical properties which can induce distinct and separate components into the afferent discharge.

**654.10**

**MORPHOLOGICAL ADAPTATIONS OF DIAPHRAGM NEUROMUSCULAR JUNCTION TO PROLONGED INACTIVITY.**


Previous models of muscle disease vary in the extent of nerve versus muscle inactivation, making it difficult to assess inactivity-induced adaptations of the neuromuscular junction (NMJ). We induced inactivation of both phrenic motorneurons and diaphragm muscle in the rat via spinal isolation (SI) at the C2 level. We then combined a three-color fluorescent photolabeling technique with 3D confocal microscopy to simultaneously visualize nerve terminals, motor endplates and muscle fiber type in SI diaphragm fibers. Compared to controls, both nerve terminal and endplate plumes (2D) and surface (3D) areas were increased on type II fibers, but not on type I fibers. The extent of overlap of pre- and postsynaptic elements of the NMJ increased on type II fibers. Terminal and endplate length lengths on type II fibers also increased. Endplate gutter depth was unchanged. These results suggest that NMJ inactivation at both pre- and postsynaptic levels leads to a phenotype-dependent expansion of the NMJ on type II fibers, perhaps in an effort to improve neuromuscular transmission. Supported by NIH grants HL34117 and HL37680.

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

465.13 INTERCELLULAR COMMUNICATION MONITORED BY PROPAGATION OF CALCIUM WAVES IN CULTURED COLONIC SMOOTH MUSCLE CELLS. S.L. Young, N. West, F. H. Mazer, and D. E. Mazur. CURE-VAM, the Neuroscience Teaching Center, Neurologic Structural Biology Group, Dept. of Physiology and Medicine, UCLA and West Los Angeles VA Med.Ctr., Los Angeles, CA 90073.

Single muscle cells respond to a light mechanical stimulus with a local increase in intracellular calcium which can propagate throughout the cell and into several adjacent cells. Smooth muscle cells were isolated from the circular and longitudinal layers of the rat colon. These cells were used to study calcium propagation by placing a fiber polished glass pipette (2.4-μm diameter) connected to an electronically controlled microinjector (Eppendorf) used to displace the plasma membrane (2 μm distance, 0.5 sec duration). The calcium concentration at the stimulus point, but if the response exceeds 100 nM above resting levels, the increase will propagate as a wave throughout the cell (intracellular velocity 13.2 μm/sec). Recovery rates to resting levels are highly variable, showing both single double time constants in the range t = 7-100 sec. When multiple cells are in contact, the wave continues to propagate into as many as 5 adjacent cells (intracellular velocity 17 μm/sec) without large degradation in amplitude. The propagation is likely the result of diffusion of a "message" substance which releases calcium from intracellular stores, and not calcium itself. The wave can propagate through cells which do not respond, and into adjacent cells which do exhibit an increase in calcium. Propagation occurs in zero calcium external solution (21 μM/sec). After treatment with thapsigargin (3 μM), a Ca2+-ATPase inhibitor which should deplete intracellular stores, the stimulated cell responded, but intercellular propagation was blocked. Treatment with U-73122, an inhibitor of phospholipase C dependent processes, including production of IP3, prevents intracellular propagation, indicating that IP3 may be the messenger molecule. Supported by NIH Grant DK49199-05.


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

LIMBIC SYSTEM AND HYPOTHALAMUS: AMYGDALA AND HYPOTHALAMUS

655.1 IONIC MECHANISMS OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION IN MAGNOCellular NEURONS OF THE HYPOTHALAMIC SUPRAOPTIC NUCLEUS. L.A. Schröder* and L.G. Takeda. Neuroscience Program and Dept. of Molecular Biology, Tulane University, New Orleans, LA 70118.

Activation of metabotropic glutamate receptors in magnocellular neurons of the hypothalamus (SON) results in a large increase in membrane conductance (Schröder and Takeda, Soc. Neurosci. Abstr. 20: 347, 1994). We have investigated the ionic mechanisms of these effects with whole-cell recordings in cortical slices (400 μm) of rat hypothalamic using the metabotropic glutamate receptor agonist (2R,5R)-trans-ACPD and selective ion channel blockers. Bath application of the Na+-channel blocker tetradotoxin (1-3μM) had no effect on the inward current or on the decrease in conductance evoked by trans-ACPD. However, when K+-channels were blocked by replacing K+ in the patch pipette with an equimolar concentration of Cs+ and by including tetraethylammonium (10-20 μM) in the bath perfusion the decrease in conductance was attenuated or blocked completely. In some cases, when K+-channels were blocked, trans-ACPD caused an increase in membrane conductance, which was antagonized by adding the Ca2+-channel blocker Co2+ (200-250 μM) to the bath. These results indicate that metabotropic-receptor activation causes a decrease in membrane conductance and an inward current in SON magnocellular neurons by closing K+-channels and by opening a Ca2+-dependent conductance. This work is supported in part by NIH NS31187.

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

Previous extracellular recording studies have suggested the existence of connections (perhaps reciprocal) between the MeA and the SON including its periventricular zone (PVN). Our intracellular recordings from single neurons investigated this potential local circuit involving the MeA, the SON and the PZ. Also studied was the nature of the input from the olfactory bulbs to MeA neurons, connections for which there is long-standing anatomical evidence. In horizontally cut slices that preserve the ventral amygdalo-hypothalamic pathway, electrical stimulation of the SON/PVN produces short latency EPSPs that are blocked by a-1 cyclohexylindan-1-carbonyl-penicillamine (cGKIα), which is an innervation to the sensory layers of the MeA. Our data also automatically activated by SON/PVN stimulation, suggesting reciprocal connections between the two regions. These MeA neurons were synaptic connections were identified with SOM/PVN stimulation, indicating convergent input into the SON. Stimulation of the SON elicited EPSPs that were blocked by the oxytocin agonist, (8-arginine)vasopressin (8-Arg, vasopressin) and a non-NO PDE to MeA neurons. These results are also consistent with recent findings of oxytocin receptors in the SON. In SON neurons, these EPSPs were blocked by the non-NO receptor antagonist, CNQX (5 μM), but both MeA neurons, displaying IPSPs in response to SON/PVN stimulation, these synaptic responses were abolished by the GABA-A receptor blocker, bicuculline. These results suggest that MeA neurons receive inputs from and project to the SON and in PZ, and that some of these inputs from the SON are oxytocinergic, probably arising from SON axon collaterals. Also MeA, like SON, neurons receive excitatory input from the olfactory pathway via the LOT. Supported by NIMH grants NS 16942.
655.3 ACTIVATION OF HISTAMINERGIC INPUTS TO PHASICALLY FIRING SUPRAOPTIC NUCLEUS (SON) NEURONS INCREASES D-FRUCTOSE-FED EFFECTS OF H1-ANTAGONISTS AND GMP. C.I. Hutton and Q.Z. Yang. Dept. of Neuroscience, Univ. of California, Riverside, CA 92521.

Activation of different input types (glutamatergic or GABAergic) in SON has shown to increase the incidence of interneuronal coupling in slices from lactating, but not virginal or male rats. Here we studied the influence of coupling on activating another neurotransmitter system, the SON histaminergic postsynaptic monoaminergic nucleus (TM) projection, which is maintained intact in our horizontally cut slices. Stimulation of the TM selectively excites phasically firing (vasopressinergic) cells, an effect which is blocked by H1-antagonists. The effects of TM stimulation and its possible downstream second messenger consequences on Lucifer Yellow (LY) dye filled and phasically firing cells in male rat SON. In unstimulated slices, LY injections into 27 cells (1 cell/SON) yielded 18 single and 18 coupled neurons. In slices in which TM was stimulated for 10 min at 10 Hz, 13 injections yielded 4 single and 18 coupled cells, a 185% increase in number of dye filled cells/injection (p < .02). LY injections into 19 cells in unstimulated and 21 injections in stimulated slices that were bathed in medium containing 10 μM pyrilamine (H1-antagonist), yielded similar numbers of coupled neurons. That this coupling effect was blocked by H1-antagonist suggested that it might be mediated by guanylate cyclase-cGMP to which H1 receptors are often linked. In a parallel study, slices were bathed in control medium or containing 0.5 mM 8-bromo-cGMP. In control medium, 15 LY injections yielded 10 single and 10 coupled cells whereas, in medium containing cGMP, 15 LY injections yielded 9 single and 32 coupled neurons, a 142% increase (p < .02) over control. Taken together, these results suggest that one mechanism mediating synaptic enhancement of interneuronal coupling involves connexon channel protein phosphorylation via cGMP-related actions. Supported by NINDS grant NS16942.

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

Histamine (HA) has been shown to function as a neurotransmitter and/or neuromodulator in the rat supraoptic nucleus (SON). Our previous studies have shown that HA can directly depolarize SON neurons by activating membrane HA receptors and this action involves Ca2+-independent intracellular processes and a reduction in K+ conductance. Here we investigated the ionic mechanisms and intracellular signal transduction involved in HA-induced prolonged depolarization.

Whole-cell recordings were obtained from SON neurons in horizontal brain slices from adult male rats. In medium containing 0 mM Ca2+, 2 mM Mg2+ and 1.2 mM TTX, with internal Ca2+ chelation (11 mM BAPTA), both application of HA induced an apparent inward current in 15/20 cells tested. The peak of inward current evoked by 10 μM HA was holding potential of -50 mV and was blocked in high-potassium (20 mM) medium, but not in medium containing 10 μM pyrilamine (H1-antagonist) or 10 μM bicuculline (GABA-A/ -B antagonists). The peak inward current in control slices was 17.7 ± 3.2 pA (Mean ± SEM). RAMP voltage tests revealed that this inward current had a reversal potential of -90 ± 13.8 mV (n = 10).

Current subtraction was performed in a current which showed little voltage dependence (e.g., Ca2+ or inward rectifier) in the medium and attenuated or abolished HA-induced inward current at membrane potentials close to -50 mV. When external Cs concentration was reduced, HA-induced inward current was 12.3 ± 1.1 pA (n = 57) cells tested. Neither inward current nor change in conductance was observed following bath application of HA in 11/12 recorded cells followed by patch pipettes containing GDP-β-S, and in 7/8 cells using pipettes containing GTP-γS. When electrodes containing the protein kinase inhibitor, H8 (0.5 mM) were used, inward current was reduced to -90 mV close to -90 mV was seen only in 1 of 11 cells tested. These results suggest that HA depolarizes SON neurons, in part at least, by inhibiting a K+ leakage current mediated by G-protein and protein kinase. Supported by NINDS grant NS16942.

655.7 SYNAPTIC INTERACTIONS BETWEEN AMYGDALO-STRIATAL AFFERENTS AND PARMALINEN AND CALRECEPTIN INTERNEURONS WITHIN THE RAT VENTRAL STRIATUM. T. D. Tasker*, J.M. Smith, and A.J. Morris*. 1 Dept Neurology, Neurosurgery, MNS, McGill University, Montreal, PC, 2. Laboratoire de Neuropsychologie, Hopital Enfant-Jesus, Laval University, Quebec City, PC.

Afferent input from the basolateral amygdala (BLA) to the ventral striatum is thought to influence volume-related responses to novel and rewarding stimuli. In this study we determined if the BLA had synaptic inputs to the ventral striatum (DS). Evidence has shown that dopamine can modulate this response and that this is related to synaptic convergence of nigral and amygdala input upon striatal projection neurons. The present study confirms that there are multiple interneuron types within the striatum, and may represent another substrate for modulation of amygdaloid inputs. Two distinct populations of interneurons, identified by their immunoreactivity to the calcium binding protein parvalbumin (PV) and calretinin (CR), have been localized within the ventral striatum. Both of these populations have been shown to receive asymmetric synapses (Lapper, et al., 1992 and Bonnet and Bolam, 1993) and are GABAergic (Kahota, et al., 1995). EE examination revealed that both PV and CR interneurons are targets of the amygdalo-striatal projection. Injections of the anterograde tracer biotinylated dextranamine were made into the BLA. Areas between the amygdalo-striatal projection and PV positive cells were analyzed by electron microscopy. Preliminary results indicate the presence of asymmetric synapses between amygdala terminals and the dendrites of PV neurons. The results presented for the first time will help support the hypothesis that these neurons mediate feed-forward inhibition of the excitatory signals arising from the amygdala.

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

The bed nucleus of the stria terminalis (BST) and adjacent central amygdaloid nuclei show striking similarities in cytoarchitecture, neurochemistry, and neuronal connections and have, therefore, been proposed to belong to one anatomical unit, the extended amygdala (Adolph et al., 1995). The vasopressin (AVP) cells of the BST and amygdala illustrate these similarities. They are similar in size and morphology; immunoreactive for estrogen and androgen receptors and for galanin. In addition, AVP expression in both amygdaloid and BST innervation is modulated by local factors such as neurotransmitters. Here we test whether AVP-immunoreactive (AVP-ir) and AVP mRNA expressing neurons are indeed found in homologous divisions of the BST and amygdala according to the present study, extended amygdala (Adolph et al., 1995) and for both AVP-ir and AVP mRNA in the BST and amygdala. The majority of these cells were found in the anterior medial BST (BSTMA), the lateral part of the medial posterior BST (HST), and the intermediate area of the lateral BST (LST). In the amygdala, the majority of the immunoreactive and mRNA labeled bed nucleus of the stria terminalis (BST), the parventromedial part of the medial amygdaloid nucleus (MeAD), and the medial part of the central nucleus (CeM). These areas are identified as each others counterparts. The distribution of AVP expressing cells within the BST and amygdala suggests that these cells may serve to provide different microenvironmental conditions within the extended amygdala. Results presented for AVP neurons in the medial BST do not differ in number. Funded by NSF grant, IBN 9421565 to G.J., and M.A grant AG-10917 to M.A.
Limbic system and hypothalamus: Amygdala and hypothalamus

655.9 LOCALIZATION OF BENZODIAZEPINE/GABA<sub>B</sub> RECEPTORS IN THE BASOLATERAL AMYGDALA OF THE RAT AND MONKEY: AN IMMUNOBIOCHEMICAL AND PHYSIOLOGICAL STUDY. M.J. Hugdahl, E.L. MacAskill, and D.P. Hugdahl, Dept. of Cell Biology and Neuroscience, Univ. of South Carolina School of Medicine, Columbia, SC 29080.

The basolateral amygdala (BLA) has a strong intrinsic inhibitory system mediated by GABA<sub>B</sub> receptors and is the main site of the anxiolytic actions of benzodiazepines. To identify the anatomical substrates in these transmitter systems, immunohistochemical techniques using antibodies to GABA<sub>B</sub> receptors were used to analyse the neuronal localization of the GABA<sub>B</sub> benzodiazepine receptor complex (GABAB/RZ) in the rat and monkey BLA.

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

The results of this study indicate that the pattern of GABAB/RZ immunoreactivity in the neuropil of the rat and monkey BLA closely resemble the distribution of benzodiazepine receptors localized in previous radioligand autoradiographic studies. The finding of intense immunoreactivity in subpopulations of nonpyramidal neurons suggests the existence of disinhibitory mechanisms which play a role in the activation of BLA, perhaps in response to behaviorally significant stimuli. (Supported by NIH Grant NS 19733).


Amygdaloid electrical stimulation in the rabbit produces short-latency activation of neurons recorded in the mesopontine peribrachial (PB) region (Pascoe et al., 1996). Given the importance of the amygdala and PB region in arousal (Kapp et al., 1992; Steriade et al., 1990), the present study was conducted to determine if activation of the PB responds to amygdala afferents. Immunohistochemical techniques were used to identify the area of PB responsive activity in the awake rabbit correlates with the state of arousal (EEG and EOG) and to examine if any such neurons are influenced by amygdaloid stimulation. To date, we have been investigating two types of extracellularly recorded auditory responsive neurons. The first, with a spontaneous rate of &lt;0.01 spikes/s, responded to a phasic burst of 1-4 spikes to the onset of auditory stimuli. The response of this type of neuron is similar to that of a POO-on burst neuron (Steriade et al., 1990) which may represent a disinhibitory mechanism in response to novel stimuli. Neither the stimulus-evoked nor spontaneous activity of these neurons showed a clear relation to the state of arousal. Consistent with previous findings, amygdaloid stimulation produced a short latency (4-13 msec) activation of these neurons. The second type, L spontaneous rate of 2.0Hz, responded with a burst of spikes which outlasted the duration of auditory stimuli and continued on to respond to an enhanced rate throughout the stimulus-induced EEG desynchronization period. In the absence of stimulus presentations, the spontaneous firing rate of these neurons was greater during periods of EOG desynchronization than during periods of EEG synchronization. Current experiments are investigating whether amygdaloid stimulation influences this type of neuron. The results suggest amygdaloid influences on PB neurons, some of which may contribute to thalamic arousal. (Supported by NSF NS 19699).

655.12 PROJECTION CELLS AND INTERNEURONS OF THE BASOLATERAL (BL) AMYGDALA: DISTINCT CONTEXTS HAVE DISTINCT PATTERNING IN CONSCIOUS BEHAVING CATS. L. Dossa<sup>1</sup>, H. Guérin<sup>2</sup> and D. Papi<sup>2</sup>, Deps. Physiol., Fac. Medicine, Univ. Laval, Québec, Canada.

Recently (Papic et al., 1992; Dossa et al., 1991), in the rat, we described the electrophysiological and morphological properties of BL neurons and identified two types of spike projection neurons (or anatomic expression). The first cell type prevailed in the BL nucleus and generated burst spikes, with 10 inter-spike intervals and may be GABAergic. The second cell type prevailed in the lateral nucleus and generated slow membrane potential oscillations when a few milliseconds depolarized. In contrast to projection cells, sensory interneurons had a higher spontaneous discharge rate (DR) and generated non-synchronous spikes during depolarizing current pulses. Here, we verified if these physiological properties were maintained in vivo and into the pattern that can be recognized extracellularly in behaving cats. The majority of the cells encountered in the BL nucleus had a low DR but were most active during slow-wave sleep (SWS). These cells corresponded to the already described spiny or burst spikes in response to cortical shocks. These findings suggest that in previous extracellular studies of the lateral nucleus, most spontaneously active cells were interneurons. Thus, these data must be reinterpreted.

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

655.13 RHYTHMIC ELECTRICAL ACTIVITY IN THE AMYGDALA: OBSERVATIONS IN VITRO AND IN VIVO. H.C. Page<sup>3</sup> and D. Papi<sup>2</sup>, 1Inst. of Physiologie, Otto-von-Guericke University, Magdeburg, Germany; 2Dpt. Physiologie, Université Laval, Québec, Canada.

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

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

655.14 SLOW INHIBITORY SYNAPTIC RESPONSES IN AMYGDALOID NEUROCELLS. L. Danda<sup>1</sup> and H.C. Page<sup>3</sup>, Instut für Physiologie, Otto-von-Guericke Universität, D-39120 Magdeburg, Germany.

Besides GABA, glycine is the main inhibitory transmitter in the nervous system. In order to investigate the contribution of glycine to synaptic transmission in the amygdala, we combined intracellular and extracellular recording techniques in slices of the guinea pig amygdala in vitro. A dense mon monochromatic staining for glycine and the GABA<sub>B</sub> and GABA<sub>A</sub>-subunits of the GABA<sub>A</sub>-receptor was observed throughout the amygdala. Local application of glycine to neurons in lateral (LA) and, as previously described<sup>1</sup>, central (CE) amygdaloid nuclei evoked a membrane hyperpolarization from rest that reversed at 300 ms. This glycine response persisted during blocked synaptic transmission, was antagonized by strychnine, and injection of kainate positively shifted the reversal potential. Local glycine administration evoked a slow IPSP in CE neurons, which reversed at -80 mV and was blocked by strychnine<sup>1</sup> in LA neurons, repetitive stimulation of the external capsule evoked a slow IPSP with similar time course but was not significantly lengthened by strychnine. This IPSP was substantially decreased during blockade of GABA<sub>B</sub> and GABA<sub>A</sub> receptors, thereby effectively inhibiting burst discharges generally observed during blocked GABAAergic transmission. These results provide evidence for the existence of strychnine-sensitive glycineergic transmission throughout the amygdala and of an additional slow IPSP in the LA nucleus with as yet defined pharmacological characteristics. Both types of inhibitory synaptic processes may contribute to epileptogenesis, GABA<sub>A</sub>-ergic transmission.

565.17

INDUCTION OF RUNNING ACTIVITY AND METABOLIC CHANGE BY THE VENTREMEDIAL HYPOTHALAMUS IN THE RAT. K. Narita1, M. Nishimura* and M. Takahashi. 1Dept. of Physiology, Fuku Medical School, Fuku 910-11, 2Dept. of Vet. Physiology and 2Dept. of Vet. Ethology, Univ. of Tokyo, Tokyo 113, JAPAN.

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

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

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

565.18

CHANGES IN LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) FOLLOWING INJECTIONS OF A GABA, ANTAGONIST INTO THE DORSOMEDIAL HYPOTHALAMUS OF RATS. T.J. Sajdyk*, A. Shekhar, J.S. Kanner, W.J. McBride and R. Chemet Dept. of Psychiatry, Indiana Univ. Med. Center, Indianapolis, Indiana 46202. Previous studies have shown that rats injected with GABA, antagonist into the dorsomedial hypothalamus (DMH) exhibit panic-like responses (Shekhar, Biol. Psychiatry, 36:748-58;1994). The purpose of this study was to identify other neuroanatomical sites possibly involved in mediating this panic-like response using the [14C]2-deoxyglucose (2-DG) autoradiographic method (Sokoloff et al., J. Neurochem. 28:897-916;1977). Rats were fitted with femoral arterial and venous catheters as well as unilateral, chronic injection cannulae into the DMH. They were injected with either 20 pmol/100 nl of BMI or artificial cerebrospinal fluid (a-CSF, 100 nl) into the DMH along with intravenous injection of 2-DG (125 uCi/Kg). The ensuing physiological response was recorded and arterial and venous blood samples were taken to determine plasma glucose and [14C] concentrations. Rats were sacrificed, their brains serially sectioned and regional LCGU values were determined. A significant increase in LCGU on the BMI injected side was seen in the DMH; lateral, paramentric, arcuate and posterior hypothalamic nuclei; CA1 region; and central gray. A significant decrease in LCGU was observed in the basolateral amygdala. These results indicate that activity in several discrete nuclei are altered during the panic-like response induced by BMI injections into the DMH of rats (Supported by MH 45362).

565.19

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

Blockade of GABA, receptors by locally injecting the antagonist bicuculline methiodide (BMI) into the dorsomedial hypothalamus (DMH) causes a panic-like response which includes increases in heart rate (HR), blood pressure (BP), respiratory rate, as well as "anxiety". Rat experiencing "anxiety" in a four-potentiometer startle test have shown increases in norepinephrine (NE) tissue levels in the DMH. Using in vivo microdialysis, the present study soughted to determine changes in extracellular NE levels following superimposition of different concentrations of BMI in the DMH. Rats were implanted with femoral arterial catheters and microdialysis probes into the DMH. After 24 hours, the DMH of conscious rats were perfused with either 100, 150 or 200 mM BMI solutions via the microdialysis probe. HR and BP responses were recorded and the extracellular levels of NE in the DMH were determined from the perfusates by using high pressure liquid chromatography. Rats receiving BMI injections showed dose-dependent increments in the extracellular NE in the DMH. These results suggest that in the DMH, increased NE release may be closely connected with GABA, receptor blockade and the associated panic-like response. (Supported by MH 45362)
656.3 HYPERGLYCEMIA DOES NOT ALTER REGIONAL CEREBRAL BLOOD FLOW (rCBF) OR REGIONAL CEREBRAL BLOOD OXYGENATION (rCBV) CHANGES IN CORTICAL ISCHEMIA (CSD) IN RATS. T.W. Lindsley, T.K. Yeh, A. Villinger, U. Drexler. Dept. of Neurology, Humboldt University Berlin, Germany.

Cortical spreading depression may play a role in the pathology of migrainous and cerebral ischemia. The extent of CSD, as an intense stimulus of energy metabolism, is as normal brain flow followed by marked increases in CBF and tissue oxygen concentration. Glucose is the main substrate of energy production in the brain. We tested the hypothesis that the changes in CBF with laser Doppler flowmetry (LDF) through the thinned bone nCBF (oxyhemoglobin HbO2, deoxyhemoglobin Hb) and oxidized mitochondrial cytochrome aa3 (CytO) were measured through the intact skull with near infrared spectroscopy (NIRS). The DC potential was monitored epidurally. CSD was induced every 15 min by topical application of KCl (10 mm) anterior from the measuring site. After 1 h of normoglycemic CSD recording, hyperglycemia was induced by i.v. glucose application (15% of glucose solution at a bolus, followed by infusion of 2%/4% glucose solution). A drop in blood pH due to hyperglycemia was balanced by mild hyperventilation. During hyperglycemia CSD was induced every 15 min for 1 h. Results are shown in table.

<table>
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<tr>
<th>nCBF and rCBF responses were not affected by hyperglycemia, which points against a role of glucose in the coupling of metabolism and CBF during CSD. (Supported by the Deutsche Forschungsgemeinschaft).</th>
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656.5 AUTOREGULATION OF CHOROID PLEXUS BLOOD FLOW DURING ACUTE HYPERTENSION. C.A. Hathaway, R D. S. W. Waller & J. L. Williams. Univ. of South Dakota School of Medicine, Vermillion, SD 57069.

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

BF was measured with radioactive microspheres in anesthetized rats. In 5 animals, MAP to the head was raised 39 ± 2 mmHg (mean ± SD) by aortic occlusion. BF to CP of the lateral ventricle was unchanged by occlusion (347 ± 148 to 387 ± 85 mmHg/min/100 g). BF to CP of the fourth ventricle (216 ± 37 to 428 ± 163 mmHg/min/100 g) was 0.045. CBF was unchanged (78 ± 13 to 25 ± 34 mmHg/min/100 g).

In other rabbits, MAP was increased 36 ± 8 mmHg by IV noradrenaline. BF to CP of the lateral ventricle was unchanged (288 ± 173 to 259 ± 98 mmHg/min/100 g). BF to CP of the fourth ventricle also was not changed (276 ± 112 to 307 ± 256 mmHg/min/100 g) by noradrenaline.

In 3 rabbits, we measured the rate of autoregulation of BF to CP of lateral ventricle with pressure increase, which increased nCBF 59 ± 17 mmHg/L. During aortic occlusion, CPBF and CBF increased 20 ± 7% and 45 ± 8% initially, and autoregulated 50% in 3.5 ± 6.3 ± 3.5 ± 1.8 s, respectively. Final autoregulation of CBF and CPBF occurred in 13 ± 8.4 ± 16 and 16 ± 8.8 ± 16 s.

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

656.6 THE DIRECT EFFECTS OF ALTERED GLUCOSE CONCENTRATION ON THE ISOLATED RAT MIDDLE CEREBRAL ARTERY (MCA). M.W.G. Snodgrass, M.V. Eckler, R. M. Kung. Dept. of Anesthesiology, Baylor College of Medicine, Houston, TX 77030.

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

Decreasing the glucose from 5.5 mm to 1.0, 1.0, and 0.5 mm for 1.5 hours each had no effect on the diameter of the arteries. When all the glucose was removed for 1.5 hours, the MCA dilated by 2.5% ± 3% ± 3% (p=0.05, n=7). When all Ca2+ was removed from the bath, the MCA further dilated by 35 ± 5% of control indicating that maximum dilation did not occur as a result of hypoglycemia alone. When glucose was restored (100 mm/g), the MCA diameters returned to control. Removal of the endothelium did not alter the response of the isolated MCA to reductions in glucose. Furthermore, inhibition of the ATP sensitive K+ channels with 10-4 M glibenclamide did not alter the response to glucose.

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

656.7 RELATIVE CONTRIBUTIONS OF GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION TO METABOLISM OF PRIMARY NEURAL CULTURES, AS ASSESSED BY MICROPHYSIOLOGY. J.A. Trafton, D.A. Allens, and R.M. Sapolsky. Biology Dept. Stanford Faculty, Stanford University, Stanford, CA 94305.

Microphysiology is a technique which measures real time changes in proton efflux rate in cultured cells or tissue slices. It is generally interpreted as measuring net metabolic rate, based on the idea that metabolic rates produce hydrogen ions as lactic and carbonic acid in proportion to activity. A problem in interpretation occurs, that glycolysis contributed 6 times as many protons/ATP as does oxidative phosphorylation. Thus, shifts in the balance between oxidative and glycolytic metabolism would change acid production, which might be detected interpreted as an altered metabolic rate. To distinguish between the glycolytic and oxidative contributions, we compared proton efflux rates measured in our in vitro cultures; and found that proton efflux was similar to the rate at which acid was produced by isolated enzyme systems. We explored whether this decrease represented a true metabolic decline. While significant declines in proton efflux at lower pH were still obvious in cultures restricted to oxidative metabolism, a shift toward oxidative metabolism was seen following acidification. At pH 6.4, glycolysis contributed less than 60% of total metabolism. Acidic reduced the rate of proton production under conditions bypassing glycolysis, with a decline to < 50% of pH 7.4 levels at pH 6.4. This suggests that while acidic decreases metabolic rate, it also shifts energy production towards oxidative metabolism. While the implications of this are unclear, it hints at the complexity of acid's metabolic effects and highlights the need for caution in interpreting microphysiometry data.
NON-CLASSICAL PATTERN OF CEREBRAL BLOOD FLOW (CBF)—PRESSURE AUTOREGULATION. B.C. Jorges*, C.R. Radovsky, A.D. Perez-Trujillo, Cerebrovascular Res. Lab., Cleveland Clinic Foundation, Cleveland OH 44195.

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

Thirty-five Sprague-Dawley rats were anesthetized (halothane, N₂O:O₂, body temperature 37°C). The first day a plastic cranial window was implanted and the animal was allowed to recover. The second day, animals were perfused with heparinized saline. The frozen block face was photographed for pH analysis prior to removing 6 consecutive or 6 microns sections. Tissues with pH < 7.4 were discarded prior to staining.

L-HM was most prevalent in regions of high glycogen content, i.e., the hippocampal regions of the dentate, radiatum, and astrocitoid layer adjacent to the granular layer of the dentate gyrus. The marked exception to this was the oxidative region in the proximal radiatum of CA3.

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

CAPILLARY MODULES IN MOUSE CORTICAL BARRIL. J. Sul, C.M. Rovain*, T.A. Woolley, Departments of Neurology & Neurosurgical, and Cell and Molecular Physiology, Washington University, School of Medicine, St. Louis, MO 63110.

A penetrating arteriole which is injected with dye and the surface venules from which dye emerges is an arteriolar domain which approximates the area of but does not coincide with a cytocromatic oxygenated area in layer I. The frozen block face analysis that an internal terminal arteriole and its capillary "module" is matched to a barrel, adult mice were fixed with mixed aldehydes. Penetrating arterioles and their terminal branches were stained by perfused Rose Bengal. The cortex was cut vertically or tangentially at 200μm with a vibromotor and barreled visualized with videomicroscopy. In tangential sections 1-2 terminal arterioles supplied each layer IV barrel. Arteriole branches in layers III were impaled with 5μm glass micropipettes; injected dye was recorded by videomicroscopy expressing from arteries into capillaries and then venules. Side branches from a vertical arteriole supplied modulated of capillaries within the barrel. The capillary modules matched to neural modules, manifest as barreis, are a substrate for control of CBF to a single cortical column during neural stimulation.

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

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

656.15 POTENTIALITY OF INTERSTITIAL ADENOSINE WITH NITRO-BENZYLTHIOinosine (NBti) AMELIORATES DELAYED CORTICAL HYPOPERFUSION FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN THE PIGLET. Y.B. Kim, T.S. Park, E.R. Gonzales, A.R. Shah, M.J. Noctis, J.M. Siddiqi, Departments of Neurology and Neurosurgical Surgery, Washington University School of Medicine, St. Louis, MO 63110.

A period of delayed hypoperfusion characterizes reperfusion (RP) following transient global cerebral ischemia. Concomitantly, reactivity of the cerebral vasculature to many physiologic and pharmacologic stimuli is impaired. We tested the hypothesis that increases in cerebral blood flow (CBF) during RP could be realized by augmenting endogenous adenosine (ADO), a potent cerebral vasodilator. Probes containing platinum wires for measurement of local CBF by hydrogen clearance were inserted bilaterally into the cortex of 9 sulfuric-anesthetized newborn pigs. One probe was perfused with the ADO transport inhibitor NBti (100g/m in mock CBF at 2 μm/min), the other with mock CSF alone. Measurements of [ADO] (μmol/L) and CBF (ml/min/100g) were determined at 20-min intervals during RP following 10 min of global cerebral ischemia induced by ligation of brachiocephalic and subclavian arteries. The table shows that both ADO and CBF were reduced during post-ischemic RP, but ADO elevation by NBti lessened the extent of delayed hypoperfusion. These results suggest that adenosine can improve post-ischemic tissue perfusability, and, in so doing, may contribute to its neuroprotective effect.


It has been focused that inflammation occurs in the CSF space after subarachnoid hemorrhage (SAH), and causes delayed vasospasm (VS). To clarify the detail of this mechanism, we analyzed cytokines (TNF, IL-1, IL-6, IL-8, IL-10, IL-1α) and soluble component of the adhesion molecules (ICAM-1, VCAM-1, ELAM-1) in the CSF and blood. Clinical materials were ten patients of severe SAH. CSF and blood were obtained 3 times simultaneously (day 3-5, 7-9, 12-14) after the onset. VS was diagnosed with angiography and neurological symptoms.

IL-6, IL-8, IL-10, IL-1α were significantly higher in the CSF than in the blood (p<0.05). IL-6 and IL-1α RA was higher in the CSF of the patients with VS (p<0.05) during day 7-9 than in the VO negative patients. Besides, IL-1α RA was also higher in the blood of the VS patients in the same period (p<0.05).

The value of the adhesion molecules were significantly higher in the blood than in the CSF at any sampling points, but no relation was found to VS. Unique behavior of IL-6, especially IL-6 and IL-1α, indicates the presence of specific inflammation in the CSF milieu. Elevation of the adhesion molecules in the blood may not contribute provocation of inflammation in the CSF. Although the sources of IL-6 and IL-1α in the CSF are still unknown, understanding of the initiation of the inflammation in the CSF following SAH may open the therapeutic window for the prevention of VS.


We studied changes in local blood flow, mast cells and blood vessel anatomy in experimental sciatric nerve ‘neuromas’ of rats (created by sectioning of the midscopic nerve and resection of the distal nerve and its branches to prevent regeneration). In previous work, we reported that rises in blood flow of early (48 hour) sciatric neuromas were mediated by local CGRP (calcitonin gene-related peptide) accumulation with vasodilation whereas persistent rises in blood flow at later times (14 days) were not associated with CGRP. In this work, we studied the vasculature of experimental neuromas (14 days) under inflating, through a femoral catheter, anesthetized rats with gelatinized India ink prior to euthanasia. Neumasia and intact contralateral nerves were then removed, frozen sectioned and stained by silver impregnation, and areas of individual perfused vessels in transverse section were measured using a computerized analysis system. Starting at 7 days following neuroma creation, multiple thin walled perfused capillaries were noted, most prominently in the loose connective tissue around the outside of the neuroma. The size and multiplicity of these perfused vessels indicated angiogenesis with vascular proliferation. Similar findings were not observed in contralateral intact nerves, sham-exposed nerves, or ‘neuromas’ at earlier time points. These microvascular changes paralleled rises in transverse area, and mast cell numbers, density and degranulation from previous work. Persistent rises in local blood flow of experimental neuromas are associated with a cellular response involving microvessels, mast cells and likely other tissue elements.


Rats given an optic nerve crush partially regain metabolic activity in retinofugal targets despite a 90% loss of retinal ganglion cells. Thus, there is either some restoration of information transfer from retina to brain or there is an adaptation such that visual function occurs with information transfer. The 2*F(2)*[C(2)OC]-glucose technique was used to determine local cerebral glucose use (LCGU) as an assessment of information transfer from retina to brain visual centers. Male adult Long Evans rats were given a mild crush (6/6 group) or cut (3/3 group) to the right optic nerve and LCGU was assessed 2, 9 or 60 days later. The LCGU procedure was determined during stimulation with a flashing strobe-light and rotating black pattern with or without photostinum (300g/Kg i.m.), a cholinesterase inhibitor known to activate retinocortical pathways. Quantitative analysis of LCGU autoradiograms according to the Sokoloff equation from rats 2 days after the cut. Phystostigmine treatment enhanced suppression of retinal driven metabolic activity and, therefore, the increase in glucose metabolism between day 2 and 9 was greater. These results show that retinofugal targets are partially restored due to an effect of an early lesion dependent depression of neuronal activity (Dischiasis).

Supported by BMEFT NBL 07, TP.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
656.19
ISCHEMIA ALTERS CEREBROVASCULAR K+ CHANNEL FUNCTION IN PIGLETS. D. W. Bujsia* and T. M. Louis. Dept. of Physiol./Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27157, and Dept. of Anat./Cell Biology, East Carolina University, Greenville, NC 27858.

Previous studies have shown that ischemia alters ion channel function in cortical neurons in piglets (APJ. 265: H389). The purpose of this study was to examine the effects of ischemia on ATP-sensitive K+ channels in cerebral arterioles. In anesthetized piglets equipped with cranial windows, we examined arteriolar responses to apamin (APK) (an ATP-sensitive K+ channel agonist) prior to and following 10 minutes of total global ischemia. Ischemia was induced by increasing intracranial pressure. Under control conditions, APK dilated arterioles by 7±1% and 24±4% at 10 M and 10 M, respectively (n = 6). Coapplication of glibenclamide (10 M) blocked dilation to APK. Following ischemia (n = 6), arteriolar dilation to 10 APK was reduced to 8±4% (P < 0.05) at 1 hr and 16±3% (P < 0.05) at 2 hrs post-ischemia. In other arterioles, pretreatment with indomethacin (5 mg/kg, iv), which blocks superoxide anion generation in cortex, prevented attenuation of ischemia of arteriolar dilation to APK. We conclude that ischemia alters function of vascular K+ channels probably via effects of oxygen radicals. Supported by HL 30260, HL 46585, & HL 50587.

656.20
DECREASED SENSITIVITY OF CEREBRAL CELLULAR ENERGY STATE TO HYPOXIA IN MICE FED A CREATINE ANALOGUE. D. Holtzman*, R. Meyers, E. O’Gorman, T. Wallman. Children’s Hospital, Boston, MA and Swiss Federal Inst. of Technology (ETH), Zurich, Switzerland.

The maturational increase in brain creatinine kinase (CK) catalyzed reaction rate in vivo is temporally associated with the appearance of close coupling of the phosphocreatine (PCr) and ATP losses in the hypoxic mouse or rat brain (Holtzman et al., Dev. Neurosci., 1993:15: 261-270). Feeding the creatine transport inhibitor, B-guanidinohippuric acid (GHPA), reduces brain CK catalyzed fluxes to the rates seen in the immature mouse (Holtzman et al., Brain Res 1989 481: 68-77) and doubles the brain cytosolic CK isozyme. Therefore, brain CK catalyzed reaction rates and isozyme activities are measured in mice fed GHPA (50 mg/kg by standard chow) for at least three weeks starting at age 35 days. Controls received the chow without GPA. 31P NMR spectra and saturation transfer measures of CK catalyzed phosphocreatine (PCr) turnover were performed, and a glibenclamide (100"mM) (to displace in Krebs buffer) was added. The approximate 24±4%o PCr/nucleoside ratios were determined in each time course for the initial period and at 12%o time points thereafter. No significant differences were obtained in GHPA-fed and control mice, although the hypoxic CK losses were increased in GHPA-fed animals (n = 12). The results suggest increased energy state thresholds to hypoxia in GHPA-fed mice. Supported by NINDS NS26371.

MOTIVATION AND EMOTION: BIOCHEMISTRY AND PHARMACOLOGY

657.1
CLOPRIMPINE PRETREATMENT BLOCKS THE DEVELOPMENT OF SCHEME-INDUCED POLYDIPSIA IN RATS. B. Epstein and J. Teitelbaum*. Department of Psychology, Bowling Green State University, Bowling Green, OH 43402.

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

657.2

Previous studies have shown that Pavlovian aversive conditioning to a tone results in increased serotonin release in the hippocampus. By contrast, hippocampal SHT release in response to a CS+ or CS- has been shown to be sensitive to the presence of contextual cues. In the current study, SHT release was measured in rats trained with a CS+ and CS- paired with a tone. These rats were then trained with the CS+ or CS- paired with a noise. The noise was presented in the familiar test chamber, but was paired with CS+ or CS- in a separate conditioning procedure. SHT release in response to the noise was found to be sensitive to the presence of contextual cues. These findings suggest that the hippocampus may be modulated by cues that are present during the conditioning procedure.
657.3 INTRALATERAL HYPOTHALAMIC INJECTIONS OF LOW DOSES OF KAINIC ACID INHIBIT LOCOMOTOR ACTIVITY. R.M. Pitser* and D. Wirsig-Archer. Department of Psychology, University of Illinois at Chicago, Chicago, IL 60607. Injections of L-glutamate into the lateral hypothalamus (LH) has been shown to elicit coordinated locomotor activity in the rat. The present study assessed the efficacy of infusing low doses of the L-glutamate analogue kainic acid and AMPA into the LH of the awake freely moving adult rat.

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

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

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

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

657.7 MESOLIMBIC DOPAMINERGIC NEURONS AND AFFECTIVE PERCEPTION - INVOLVEMENT OF AMPHETAMINE ACCUMBENS INTERACTIONS. A. Louis* and C. Besse, INSERM U 259 - Université de Bordeaux II - Demeure de Carriere - 33077 Bordeaux Cedex - France.

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

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

The DAergic response was increased in the CS an increase of about 120% above the baseline was observed in the control group + PBS whereas DA signal decreased rapidly of about 25% in the experimental group + PBS in the control group + TTX no significant change was observed whereas in the experimental group + TTX the olfactory stimulus induced a rapid increase of about 50% above the basal level.

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


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

657.6 THE NEUROCHEMICAL ORGANIZATION OF EMOTIONAL TOLERANCE IN AN AVIAN MODEL. "G. Beveridge* P.J. Walkey* and B. Minwitz, Institute for Zoology, University of Salzburg, Austria.

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

657.8 BLOCKADE OF NON-NMDA RECEPTORS IN THE NUCLEUS ACCUMBENS SHELL AND VENTROMEDIAL STRIATUM ELICITS INTENSE FEEDING IN RATS. C.S. Maldonado* C.J. Lecacov*, J.S. Barry, B.J. Miller, University of Wisconsin-Madison Medical School, Madison, WI 53705.

The present studies investigated the role of glutamatergic inputs within the ventromedial striatum in ingestive behavior. Local microinjection of the AMPA/Akainate antagonists DNQX, CNQX and NBQX (0.25, 0.75 μg bilaterally) into the shell subregion of accumbens resulted in an immediate, pronounced feeding response (p<.001). A dose of 2 μg did not affect feeding. Infusion of equimolar doses of DNQX into the core subregion, 0.8 mm lateral to the shell site, did not induce feeding. The feeding response proved to be pharmacologically selective in that infusion of NMDA antagonists AP-5 and MK-801 into the accumbens shell did not affect feeding behavior. Systemic pre-treatment with naloxone did not affect the feeding response to DNQX infusion into the shell, however, feeding was reduced upon systemic administration of D1 and D2 antagonists. Furthermore, concurrent injection of the GABA agonist muscimol into the lateral hypothalamus, a major target of shell efferents, abolished the response. A mapping study of the ventral striatum indicated that feeding was elicited from ventromedial, posterior dorsal, anterior dorsal, and dorsomedial sites following DNQX infusion. However, a significant feeding response was obtained from the ventromedial caudate, just posterior to the accumbens. The most sensitive site thus far examined was the posterior accumbens shell. Animals infused with a low dose of DNQX (50 ng) into this region showed a striking feeding response. These data suggest that AMPA/Akainate receptors in the accumbens shell, and perhaps neighboring ventromedial striatal regions, play a critical role in ingestive behavior, perhaps via the lateral hypothalamus.
657.9
NMDA ANTAGONIST AP5 BLOCKS THE ANXIOLYTIC EFFECT AND LACTATE INDUCED PHYSIOLOGICAL AROUSAL CAUSED BY CHRONIC CONCUBINE PROXHIBITION. IN THE DORSOMEDIAL HYPOTHALAMUS OF RATS. S. Shekar

The present study was conducted to test if the panic-like state caused by chronic concubine prohibition of GABA inhibition in the dorsomedial hypothalamus (DMH) is due in part to increased N-methyl-D-aspartate (NMDA) receptor mediated excitatory drive and can be blocked by acute injection of the NMDA antagonist AP5. Rats were fitted with femoral arterial and venous catheters and baseline response in the social interaction (SI) test of anxiety as well as heart rate (HR) and blood pressure (BP) responses to sodium lactate injections (10 mg/Kg i.v.) were obtained. Under anaesthesia, chronic injection cannulae were connected to a detachable Alzet infusion pump filled with the GABA synthesis inhibitor l-alginic acid (L-3, 5 mg/Kg/Lu/hr) and implanted into the DMH. After 4 days, rats were tested in SI and with i.v. lactate injections to establish the development of anxiety and increased HR and BP responses to lactate. On days 6 and 7 of L-3 injections into the DMH, after injection of either artificial cerebrospinal fluid (a-CSF) or the NMDA antagonist AP5 (100 pmol/ 100 nl) into L-3 infusion site in the DMH in random order, rats were once again tested in SI and with i.v. lactate injections. Injecting AP5 and not a-CSF into the DMH significantly blocked the chronic GABA dysfunction induced anxiolytic effect and lactate response, suggesting an increased NMDA mediated excitatory neurotransmission in this region (Supported by MH 45362).

657.10
MUSCARINIC RECEPTORS IN THE NUCLEUS ACCUMBENS MEDIATE A REINFORCING EFFECT. S. Heimsta, B. S. Glaser, J. M. Murphy, & W. J. Milde. Dept. of Psychiatry, Purdue University, West Lafayette, IN 47907.

The nucleus accumbens (NAC) has been known to be an important brain region for participation in reinforcement processes. Thus far, few studies have examined the possible involvement of the cholinergic NAC system in reinforcement processes. Therefore, the present study was undertaken to determine, using an intracranial self-administration technique, whether muscarinic acetylcholine receptors, present in the NAC, are involved in the reinforcing effect of nicotine. The results indicate that nicotine-induced self-administration in rats is mediated through muscarinic receptors and that both nicotine and the muscarinic antagonist atropine produce reinforcing effects. A unilateral 22-guage cannula was stereotaxically implanted in adult female Wistar rats and aimed at the NAC. After at least one week of recovery, animals were given the opportunity to self-administer a drug injection (n = 6) or its vehicle (n = 5) into the NAC. The testing chamber was equipped with two levers; the depression of one lever (active) delivered 100 nl of a 3.28 mg/ml solution of nicotine (n = 6) or its vehicle (n = 5) into the NAC. The number of responses on the active and inactive levers for the carbachol solution was significantly higher (p < 0.05) than that for vehicle animals. Carbachol subjects responded on the active lever significantly more (p < 0.001) than they did on the inactive lever (907 ± 154 n/s vs. 465 ± 154 total responses for active and inactive levers, respectively). Vehicle animals did not discriminate between the two levers (274 ± 7.1 n/s vs. 226 ± 6.4 total responses for active and inactive levers, respectively). The present results suggest mediation of muscarinic receptors in the NAC produces a reinforcing effect (IAA 0.9619, AA (R555).

657.11
BOVINE GROWTH HORMONE (bGH) TRANSGENIC MICE DISPLAY INCREASED SPONTANEOUS LOCOMOTOR ACTIVITY AND LOCOMOTOR STIMULATORY RESPONSE TO D-AMPHETAMINE. B. Salom

Recent clinical and animal data indicate a role for growth hormone (GH) in mechanisms related to anhedonia/hedonics, psychic energy and reward. Thus, GH substitution in GH-deficient patients has resulted in positive effects on psychic energy and drive. Moreover, GH-deficient patients are less frequent smokers than age-matched controls, whereas bovine growth hormone (bGH) transgenic mice show increased preference for ethanol and nicotine over water in free-choice models. In the present experiments we have investigated whether bGH transgens and controls differ in spontaneous locomotor activity (LMA), acute nicotine sensitivity, and a behavioral response related to brain dopamine (DA) and reward mechanisms, as well as in LMA response to drugs of abuse known to interact with brain DA systems. The mice were tested for LMA in activity boxes once a week for five weeks. When first exposed to the test-apparatus bGH animals displayed significantly more LMA during the first 30 min of the recording period. As the test progressed and all marked positive effects of bGH on LMA, bGH transgenic mice were significantly less stimulated by d-amphetamine than non-transgenic controls during the entire period. At the 5th test occasion the animals received ethanol (2.5 g/kg, i.p.) and nicotine-di-tartrate (0.5 mg/kg, s.c.), respectively, and tendencies for larger LMA responses in the bGH transgenics were observed. In conclusion, bGH transgenic mice display more spontaneous LMA than non-transgenic controls, and, possibly, also a disturbed habituation process. The studies suggest that the behavioral differences observed are related to differences in brain DA systems; indicating a hypersensitivity of these systems in bGH transgenics. These findings may be of relevance for the reported psychic effects of GH in humans.

657.13
SULPHITE ATTENUATES CONDITIONED PLACE PREFERENCE FOLLOWING SEXUAL BEHAVIOR IN FEMALE SYRIAN HAMSTERS. B. F. Atwood and M.A. Loos, Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

The ability of the dopamine D1 antagonist, sulpiride, to prevent the acquisition of a conditioned place preference was examined in female hamsters. Females were treated with one of several doses of sulpiride (12, 18, or 24 mg/kg body weight) prior to sexual encounters with a male in a conditioned place preference apparatus. Preliminary results indicate that sulpiride-treated females, like vehicle-treated females, exhibited near maximal levels of sexual behavior (lordosis) throughout the conditioning sessions. Despite showing high levels of lordosis, the sulpiride-treated females did not show evidence of conditioned place preference. Vehicle-treated females replicated earlier findings of conditioned place preference induced by sexual behavior in female hamsters. These results suggest that conditioned place preference in a useful means for probing the appetitive components of female sexual behavior, and that D1 receptors are involved in this appetitive process.

This research was supported by a grant from the National Science Foundation (IBN-9412543).

657.14
ROLE OF GLYCINE/NMDA RECEPTORS IN THE DORSAL PERIAQUEDUCTAL GRAY OF RATS UNDER THE INFLUENCE OF ANXIOLYTIC SELECTIVE DRUGS. R.P. Cabrelo
d, B. and M.M. De Sousa, Depto. de Farmacologia, CEB. UFSC, Florianopolis, SC, 88040-900, Brazil.

The midbrain periaqueductal gray (PAG) coordinates responses of an animal facing aversive stimulus. Excitatory amino acid (EAA) neurotransmission at the dorsal PAG (DAG), may mediate distinct behavioral performance in animal models of anxiety. The purpose of this investigation was to explore the effects of different PAG drugs on anxiety-related behavior in rats. The behavioral performance of rats anxiolysis selective drugs effects. Assessment of anxiety-related behavior was performed in male hooded rats with chronically implanted cannulae, aimed to the DAG. Fifteen min after receiving an IP injection (1 mg/kg) of diazepam (DZP; 0.375, 0.75 mg/kg), pentetrazol (PTZ; 15, 30 mg/kg) vehicle, the rats received a microinjection (0.4 µl) of ACSF, glycine (GLY 60 nmol) orCartItem (78.2 µmol/kg) into the DAG. Five min after receiving both treatments the animals were placed in the elevated plus-maze task for a 5 min observation. In experiment 1, DZP increased (p < 0.05) both the effects of OAE (60 ± 5.8) and the Scime spent (TSO; 36 ± 9.9) on open arms when compared to baseline levels (OAE = 31 ± 6.2; TSO = 63 ± 2.0). These DZP anxiolytic effects were reversed after IP microinjection of GLY (OAE = 53 ± 2.8; TSO = 9.6 ± 3.3). In experiment 2, the treatment with PTZ decreased the OAE (10 ± 6.9) and the TSO (2.6 ± 2.0). These PTZ anxiolytic effects were reversed after IP microinjection of GLY (OAE = 53 ± 2.8; TSO = 23 ± 3.3). The results suggest that the behavioral effects of anxiolytic compounds depend on baseline glutamatergic DAG activation. Also these results strengthens the hypothesis of a modular role of EAA systems in anxiety.

Supported by: CNpq and CAPES
657.15
COMPARISON OF THE EFFECTS OF D1 AND D2 Dopamine Receptor Antagonists on the Response-Reinstating Properties of Food Reinforcement
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The current experiment examined the ability of selective dopamine D1 and D2 receptor antagonists to prevent the reinstatement in operant responding produced by a single reinforced trial in the midst of extinction. Hungry rats were trained to run a straight-away for food reward during single daily trials. After 12 reinforced trials, the operant was extinguished during consecutive daily non-reinforced trials. Ss were then injected 30 min prior to a single Treatment trial with one of four doses of either D1 antagonist, raclopride (0.0, 0.1, 0.5, and 0.25 mg/kg IP) or the D2 antagonist SCH 39166 (0.00, 0.02, 0.01, 0.005 mg/kg IP). Twenty-four hours later, a Test trial was conducted in an unbiased runway. On Treatment day, a single reinforced trial was sufficient to reinstate operant running 24 hrs later (i.e. on Test day). While raclopride on Treatment day dose-dependently attenuated the response-reinstating effects of food as measured on the next trial/day, interpretation of SCH 39166 data was made difficult because it produced no consistent dose-response effects and it attenuated performance of a motor control group. In a separate experiment, neither drug produced reliable reductions in spontaneous locomotor activity measured 24 hrs post-injection. Together these data suggest a clear role for D2 receptor subtype in food reinforcement, but the role of the D1 receptor remains unclear.

657.17
THE EFFECT OF SEROTONIN AND Dopamine Reuptake Inhibitors ON Dorsal Raphé Self-Stimulation Thresholds
University of Ottawa, Ottawa, Canada K1N 6N5.

Male Sprague-Dawley rats were implanted with monopolar electrodes in the dorsal raphe nucleus (DR). Using a descending method of limits, self-stimulation frequency thresholds were obtained before and after acute intraperitoneal injections of cocaine HCl (20mg/kg), paroxetine (10mg/kg), and paroxetine (10 mg/kg or 20 mg/kg) plus cocaine (20 mg/kg). Injection of cocaine, a mixed serotonin/dopamine reuptake inhibitor, led to a 37% drop in frequency threshold. Injection of paroxetine, a selective serotonin reuptake inhibitor, did not decrease threshold. However, paroxetine (20mg/kg) injected 30 min prior to cocaine led to a 69% drop in threshold. Results suggest that increasing serotonin levels may enhance the ability of dopamine agonists to facilitate self-stimulation in the DR.

657.18
DISSOCIATION OF REWARD AND PERFORMANCE CHANGES FOLLOWING ICV MICROINJECTIONS OF NEUROTENSIN.
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Several lines of evidence show that neurotensin (NT) modulates central dopamine (DA) functions. When microinjected into the ventricle, for instance, NT produces neuroleptic-like effects and inhibits the increase in locomotion produced by DA receptor activation in the nucleus accumbens. Such an inhibitory effect of NT on DA-induced locomotion contrasts with its stimulatory effect on DA cell firing and on DA metabolism and release and implies that this postsynaptic action of NT is strong enough to counteract its action on DA impulse flow. Another possibility is that the inhibitory effect of ICV NT on DA-induced locomotion results from, or is potentiated by, its non-specific effects as NT is known to induce hypotension, muscle relaxation and hypothermia. In this study, we used the curve-shift method as applied to brain stimulation reward to determine whether the suppressant effect of ICV NT on a DA-dependent behavior is specific or is due to a general decrease in motor capability. Male rats implanted with a stimulating electrode in the centromedial nucleus of the ventral tegmental area (VTA) were trained to lever press for trains of 200 msec cathodal pulses of fixed intensity and variable frequency. Once responding was stable, the effects of three doses of ICV NT (3, 10 and 30 μg/16 μl) on the function relating the rate of responding to the stimulation frequency was determined, just before, and for 95 min after the injection. Results obtained demonstrate a clear dissociation between performance and reward changes. At every dose tested, NT produced a significant suppression of maximal responding, reflecting a decrease in motor capability. At the highest dose, a decrease in reward threshold occurred 30 min later than the onset of the suppression of responding and outlasted the later effect by at least 30 min. These results suggest that ICV NT suppresses motor capability, an effect that may interfere with the behavioral expression of an increase in DA-dependent functions. Supported by a grant from the Medical Research Council of Canada.

657.19
STRESS-INDUCED SENSITIZATION, BUT NOT FACILITATED LEARNING, IS CHOLINERGICALLY-MEDIATED. 
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Acute exposure to an inescapable stressor of restraint and intermit-tent tail-shock enhances sensory reactivity and independently facilitates acquisition of the classically conditioned eyelash response in the freely-moving rat. Because the same stressor increases acetylcholine (ACH) release into the hippocampus, amygdala, and prefrontal cortex, we tested the hypothesis that either one or both of the stress-induced behaviors was cholinergically mediated. Rats (6/group) were treated with the muscarinic antagonist scopolamine (5 mg/kg) or vehicle 30 min prior to the stress and no stress condition. 24 hrs later, they were exposed to 10 white noise stimuli (85 db) and trained with paired and unpaired stimuli consisting of a white noise conditioned stimulus overlapping and coterminal with a periodical shock unconditional stimulus to the eyelid. Preventing activation of muscarinic receptors during the stressor prevented the sensitization (p = 0.02) but not the facilitated learning (p = 0.99). Additional data suggest that the effective ACH is not originating in the septum; severing the fornix (n = 12) did not prevent the enhanced reactivity to auditory cues. These results suggest that the sensitization effect is cholinergically-mediated, although not via septal inputs to the hippocampus. Moreover, these results pharmacologically dissociate the stress-induced sensitization from the facilitation of associative learning. [Whitehall and McDonell-Pew Foundations and ONR support to TJS]

A circadian pacemaker in the eye of Aplysia generates a rhythm of spontaneous nerve impulses. We have found that this system produces the phase shifting agents light and 5-HT that modify the synthesis of oscillator proteins at the level of translation and perhaps transcription as well. Using 2-D gel electrophoresis, we previously searched for putative protein products in the eye extract that were modified by treatments with light and 5-HT. We found that light treatments to isolated eyes at CT 18:24 increased incorporation of [3H]leucine into BIP (a molecular chaperone in the endoplasmic reticulum) whereas decreasing incorporation of label into porin (a large conductance channel in mitochondrial and other membranes). 5-HT treatments to eyes at CT 6-12 increased incorporation of label into BIP and porin.

To investigate the effects of phase shifting treatments on Bip and porin at the transcriptional level, we first obtained their cDNAs. Clones of Aplysia Bip were obtained from Kuhl et al., and clones of porin were obtained by screening as Aplysia ganglia cDNA library using a clone from human porin. A full-length cDNA of 282 amino acids, obtained from the Aplysia library, was 67% identical to human porin. Riboprobes complementary to coding regions of Bip and porin were used in ribonuclease protection assays to measure their mRNA levels. Light and 5-HT administered to isolated eyes had opposite effects on the levels of mRNAs of Bip and porin. The effects of light and 5-HT on Bip and porin mRNAs correlated with their effects on proteins except for the action of light on Bip mRNA and protein. Light at CT 18:24 decreased levels of mRNAs of Bip (562±2%, n=4) and porin (37±6%, n=4), while 5-HT at CT 18:12 increased levels of mRNAs of Bip (50±20%, n=7) and porin (45±14%, n=6). These results indicate that light and 5-HT may phase shift the ocellar circadian oscillator by regulating levels of Bip and/or porin mRNAs.


Division of Pediatric Cardiology, Dept. of Pediatrics, Rainbow Babies & Children Hospital, Case Western Reserve Univ., Cleveland, OH 44106, and Dept. of Genetics, Case Western Reserve Univ., Cleveland, OH 44106.

The basis of the circadian pacemaker in the SCN (suprachiasmatic nucleus) remains speculative. Our previous study confirmed robust expression of highly polyubiquitinated neural cell adhesion molecule (PSA-NCAM) in adult rodent SCN. PSA-NCAM, capable of modulating contact-dependent cell-cell interactions, is involved in a variety of developmental events and in adult neuroplasticity. To explore the role of PSA-NCAM in the SCN circadian clock, we assessed circadian locomotor activity rhythms under various photoperiodic regimens in the mice with the deletion of the major PSA-NCAM-expressing region of NCAM-180. The results showed that NCAM-180 deficient mice (n=15), with total loss of NCAM-180 and PSA in the SCN had a shorter endogenous free-running period (17.1±0.9 h) and a longer activity time (n=18,24.0±2.2 h) under constant darkness (DD) than wildtype (n=12, 23.5±0.05 h). P<0.05, n=15, 18.40±8.7 h). By wk 4 of DD the majority of mutant mice exhibited a desynchrony activity rhythm. Also, the phototransient of locomotor activity in NCAM-180 deficient mice which was uniphasic under the initial 12L:12D photoperiod (LD), was (in contrast to the wildtype) disrupted after return to LD following DD exposure. Collectively, these findings are evidence that PSA-NCAM, and possibly NCAM-180, are important to the generation of circadian rhythms and may help maintain the coherence of these rhythms.

565.5 IDENTIFICATION OF PINAL SPECIFIC GENES IN RAT BY DIFFERENTIAL DISPLAY PCR. X. Wang, Michael J. Brownstein, and W. S. Young. III. Lab. Cell Biology, NIMH, NIH, Bethesda, MD 20892.

In the mammalian pineal, the circadian production of melatonin is controlled by the rhythmic expression of acetyl-CoA: serotonin N-acetyltransferase (SNAT). Acetyl-CoA is a limiting step in serotonin metabolism. Intraperistaltically injected in intact rats stimulate the expression of SNAT or SNAT regulator(s) transcriptionally or translationally. Various cloning methodologies have failed to find a chicken SBAT that is more than 1 ribosomal subunit of the major PSA-NCAM-expressing region of NCAM-180. The results showed that NCAM-180 deficient mice (n=15), with total loss of NCAM-180 and PSA in the SCN had a shorter endogenous free-running period (17.1±0.9 h) and a longer activity time (n=18,24.0±2.2 h) under constant darkness (DD) than wildtype (n=12, 23.5±0.05 h). P<0.05, n=15, 18.40±8.7 h). By wk 4 of DD the majority of mutant mice exhibited a desynchrony activity rhythm. Also, the phototransient of locomotor activity in NCAM-180 deficient mice which was uniphasic under the initial 12L:12D photoperiod (LD), was (in contrast to the wildtype) disrupted after return to LD following DD exposure. Collectively, these findings are evidence that PSA-NCAM, and possibly NCAM-180, are important to the generation of circadian rhythms and may help maintain the coherence of these rhythms.

565.6 CLONING OF THE MOUSE MEL1-MELATONIN RECEPTOR GENE. Alfred L. Roca* and Steven M. Repetto. Laboratory of Developmental Chronicology, Mass. General Hospital, and Program in Neuroscience, Harvard Medical School, Boston MA 02114.

Recently a high-affinity melatonin receptor, designated the Mel1- melatonin receptor, was cloned from mammals (Reppetto et al., 1994; Neurons 13:177). Defining the structure of the murine receptor gene is a necessary step for generating transgenic animals. Degenerate primers were designed using regions conserved among the mouse Mel1-Melatonin receptor (Mel1-Melatonin) mRNAs. PCR of mouse genomic DNA yielded a 466 bp fragment that was 94% identical at the amino acid level to the rat and Drosophila 1-Melatonin receptors. In situ hybridization of adult C57BL/6J mouse brain using the (RT-2) which expresses the Mel1-Melatonin receptor. Northern analysis of poly(A)+ RNA indicated a transcript length of ca. 1.5 kb. RT-PCR was used to generate the full-length coding region (1059 bp) of the receptor, which showed 84% amino acid identity to the human Mel1-Melatonin receptor. RNAse protection analysis, 5' and 3' RACE, and screening of murine genomic library revealed that the receptor gene consists of 2 exons divided by a large (>8 kb) intron. The 3' untranslated region is 444 bp long, and includes the poly(A) signal. Southern analysis of a genomic DNA library suggests that a large transcription start site is located at 100 bp upstream of the initiation codon.
658.7
MELATONIN ACTION VIA PROTEIN KINASE C IN THE SCN OF THE RAT. A.E. Hunt, A.J. McArthur, M.U. Gillette*. Neuroscience Program and Departments of Physiology and Cellular and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Production of the pineal hormone melatonin occurs only at night, or during the dark phase of the subjective light-dark cycle, and is controlled by a variety of mechanisms. We have used aldosterone to determine the protein kinase C (PKC) pathway in the pineal gland.

658.8

We (Brain Res, 1992; J Neurosci, 1995) and others (Yama et al., J Neurosci, 1993) have shown that Fos-LI occurs in pontine areas in response to cholinergically-induced REM sleep (RUMCs). Since pontine cholinergic mechanisms have been implicated in REM sleep generation, in this present study we examined whether pontine cholinergic neurons show Fos-LI in association with REMCs.

Sleep was recorded from cats given pontine microinjections of either saline (n=2; 0.25μl) or carbachol (n=3; 2 μg/0.25μl). Carbachol treated animals had REMc (27-40 min) and were sacrificed (nembutal) at end of REMc. In saline treated animals, very few Fos-LI cells were found in the pons. None of these cells were colocated in LTD-PPT cholinergic neurons. On the other hand, the animals with REMc showed a consistent pattern of Fos-LI cells in the dorso lateral pons. In the REMc animals, a subpopulation of the LTD-PPT cholinergic cells were also Fos-LI positive (contralateral= 10.17%; ipsilateral= 11.5%).

We found that only a subpopulation (10.64%) of LTD-PPT cholinergic neurons were also Fos-LI positive. It is interesting that single-unit studies have also found that only a subpopulation of neurons recorded in the LTD-PPT are "REM-on". Thus, even in a phenotypically similar population (i.e., cholinergic) only a subset are differentially affected during REM sleep, as assessed by electrophysiological or molecular techniques.

658.9

Immobilization stress, cold water swim, and chronic or acute drug treatments alter c-fos, preproencephalin (PPE) and dynorphin (DYN) mRNA levels. c-Fos is known to induce the expression of both PPE and DYN. In the present study, changes in the expression of these messages were investigated using the platform technique of REM sleep deprivation. Rats were placed on either large platforms (LP) or small platforms (SP) for 1 and 5 days, while control animals were kept in dry cages throughout the experimental period. Northern blot analysis showed decreased levels of c-fos mRNA in the cortex and striatum of platform animals compared to rats maintained in dry cages. In contrast, PPE mRNA levels were increased in the cortex and striatum of platform animals. DYN mRNA levels were unchanged.

The differential expression of messages whose protein products have been associated with various aspects of the transcripational cascade described in other systems, suggests that a similar analysis of events may play a role in this experimental paradigm.

658.10
CHANGES IN c-FOS AND CHAT mRNA LEVELS IN SPECIFIC RAT BRAIN REGIONS DURING NORMAL SLEEP. M.A. Greco1, G. Pendergast, J.L. Neuros, P. Shiromani, J.L. Neuros2. Harvard Medical School, VA Medical Center, West Roxbury, MA 02132, and New England Medical Center, Boston, MA 02111.

Studies in this laboratory have investigated neuronal mechanisms underlying sleep-wake states. These studies have also shown an increased number of c-fos immunoreactive cells in a subpopulation of pontine cholinergic neurons in response to drug-induced REM sleep. We have now begun to examine molecular events associated with physiological sleep. Initial experiments using northern blot analysis indicated a more sensitive assay was required to investigate possible transcriptional regulation mechanisms of gene expression in this system. In the studies described here, c-fos and Chat mRNA levels from discrete brain regions were analyzed across normal sleep states. Rats were sacrificed during waking, slow wave sleep or REM sleep. Total RNA was extracted from punch biopsies of the medial PFR, DRN, LC and LDT regions of the pons as well as hypothalamic areas. Messenger RNA levels were analyzed by quantitative RT/PCR. The results indicate that c-fos and Chat messages are differentially and variably expressed. These studies show the utility of RT/PCR to analyze low abundance messages in specific areas of the brain.

658.11
CHANGES IN TYROSINE HYDROXYLASE mRNA LEVELS ASSOCIATED WITH THE PLATFORM TECHNIQUE OF REM SLEEP DEPRIVATION. D. Hamann1, L. Ramirez, R.W. McCarley, and P. Shiromani1. Dept Psychiatry, Harvard Medical School; VA Medical Center, West Roxbury, MA 02132.

A number of experimental techniques have been shown to induce the expression of tyrosine hydroxylase mRNA in locus coeruleus. We studied alterations in TH mRNA using a paradigm that also produces REM sleep deprivation in rats.

Rats were placed on large platform (LP) and small platform (SP) for one and five days and compared with controls kept in dry cages. Changes in TH mRNA were analysed by northern analysis of total RNA isolated from pons, locus coeruleus and ventral tegmental area. Preliminary results showed that compared to dry cage controls there was no significant change in TH mRNA in LP rats. Whereas in SP animals there was a significant increase in TH mRNA after one day which drops to control levels after five days. It will be important to examine the changes in TH mRNA in discrete pontine nuclei using in situ hybridization.

These data indicate that TH mRNA levels are changed with the pedestal treatment.

658.12
ULTRA VIOLET-SENSITIVE RETINAL PHOTORECEPTORS MEDIATE LIGHT-INDUCED PHASE-SHIFTS AND FOS EXPRESSION IN RAT SUR Pra R RACHASMATIC NUCLEUS. S. Amir* and B. Robinson, Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada. Mammalian circadian rhythm is generated by a pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) and are entrained by photic stimuli that are carried to the SCN in a direct retinal projection, the retinohypothalamic tract (RHT), and in an indirect projection from the intrageniculate leaflet (IGL), the geniculohypothalamic tract (GHT). The retinal photoreceptors underlying entrainment, and that are thought to be distinct from those involved in vision, have not been identified. In rodents, photic stimuli that entrain circadian rhythms induce expression of the transcription factor Fos in SCN cells innervated by the RHT and GHT. Here we report that in rats ultraviolet light pulses known to excite the retina electrophysiologically induce a pattern of Fos expression in the SCN that corresponds to the known pattern of distribution of RHT and GHT terminals in the SCN region. The effect of ultraviolet light on Fos expression is phase dependent and appears to be mediated by a retinal mechanism bearing characteristics of a specific ultra violet receptor. Because we find in addition that ultraviolet light pulses that induce Fos expression in the SCN are sufficient to induce phase shifts in circadian rhythms, we conclude that the retinal photoreceptors that mediate the effect of light on rodent circadian pacemaker are ultraviolet sensitive.
658.13

**DAY-NIGHT DIFFERENCE IN THE NUMBER OF FOS-IMMUNOREACTIVE NEURONS IN THE SUPRACHIASMATIC NUCLEUS OF FETAL SHEEP.** S. Brent, L. Eren*, L. Wine*, D. Walling*, Department of Anatomy & Cell Biology, University of Melbourne, Parkville, Victoria 3052, Australia; *Department of Physiology & Department of Psychology, Monash University, Clayton, Victoria 3168, Australia.

Fetal sheep exhibit several behavioural and hormonal diurnal rhythms in late gestation. It is unclear whether these rhythms are controlled directly by the fetal suprachiasmatic nucleus (SCN) or result from diurnal changes in maternal hormones. FOS immunoactivity (FOS-ir) was utilised as a marker of neuronal activation to determine if SCN neurons are active during gestation. Pregnant ewes (n=28) were exposed under a 12 h light-dark cycle prior to administration of an overdose of sodium pentobarbitone at either 1200 h or 0300 h. At 1200 h in the 75 d fetus (term=146 d) a few FOS-ir neurons were observed while in the 90, 105, 117 and 138 d fetuses many FOS-ir neurons were present throughout the nucleus. However fetuses killed at 0300 h showed no difference between operated and control fetuses, indicating that retinal input is not essential for entraining the fetal SCN. Another possibility is that a chemical messenger which undergoes diurnal fluctuations in the mother may contribute to a diurnal signal to the fetal SCN.

658.15

**FOS IMMUNOREACTIVITY IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE DIURNAL RODENT ARVICANTHIS MAMMALIS.** Catherina Katma, Cheryl van Stolk, Laura Smale, Dept. of Psychology/Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The suprachiasmatic nucleus (SCN) regulates circadian rhythms in all mammals examined to date and has been studied extensively in nocturnal species. Little is currently known about differences in the neural mechanisms controlling rhythms in nocturnal and diurnal animals. These functional differences may exist either within or between species, or among different elements of the neural network. We used Fos as an index of SCN function to explore the hypothesis that mechanisms within the SCN are coupled in one way to the LD cycle in nocturnal animals and are coupled oppositely in diurnal animals. Fos is a phosphoprotein which interacts with DNA to regulate gene transcription. The number of Fos immunoreactive (Fos-ir) cells in the SCN of a nocturnal rodent, Arvicanthis mammalis, was examined. Our study included 36 adult mice sacrificed at 6 time points around a 12:12 LD cycle. We found that Fos-ir was significantly higher in the SCN of A. mammalis: During the light part of the cycle there was an increase in the number of cells expressing Fos-ir whereas during the dark the number of Fos-ir cells was lower.

Thus, the rhythm of Fos-ir in the SCN is similar in the nocturnal laboratory rat and the diurnal species A. mammalis: in a 12:12 LD cycle; however, it remains possible that temporal differences of Fos-ir occur in different subsets of SCN cells.

658.17


Circadian rhythms of mammals are timed by an endogenous clock with a period of about 24 hours located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Light synchronizes this clock to the external environment by directly activating the circadian clock through the pineal gland. The light signal can also be modulated by light-activated cellular signaling pathways such as the ERK/p38 MAPK pathway, which regulates the activity of CREB (cAMP response element binding protein), a key regulator of gene transcription and circadian rhythmicity. In this study, we examined the effects of light, glutamate, and nitric oxide on CREB phosphorylation and gene transcription in the SCN of adult rats. To determine the circadian regulation of CREB phosphorylation, rats were exposed to continuous light for 6 hours, and then to a 12:12 light-dark cycle for 6 hours. Rats were sacrificed at 2-hour intervals throughout the cycle, and the phosphorylation status of CREB was determined by Western blotting. Our results showed that light exposure led to a significant increase in CREB phosphorylation, while glutamate and nitric oxide had no significant effect on CREB phosphorylation. These findings suggest that the circadian clock regulates CREB phosphorylation in the SCN through direct light-mediated signaling pathways, which may be important for maintaining circadian rhythmicity in response to changes in the external environment.

658.18

**LIGHT-INDUCED AND CIRCADIAN EXPRESSION OF fosB, c-fos, junB and c-jun IN THE HAMSTER SUPRACHIASMATIC NUCLEUS.** M. E. Guidet*, B. Euskirchen*, A. Gobin, J. Labrie, Department of Psychology, Pharmacology, Dalhousie University, Halifax NS, Canada B3H 4J1.

The suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is the site of an endogenous pacemaker that is responsible for timing expression of daily rhythms. The circadian rhythmicity of this pacemaker is synchronized by light, and a molecular correlate of photic entrainment is the rapid and transient induction of various immediate early genes (IEGs) in SCN cells. These IEGs encode for, among other things, transcription regulatory factors such as Fos and Jun family proteins. The anatomical pattern of c-fos induced by light is similar to the pattern of retinohypothalamic fiber terminals in the SCN. Fos and Jun proteins could be regulating transcriptionally the expression of genes responsible for the timing of the intracellular circadian pacemaker.

To study the role that other IEGs could be playing in the SCN, we have characterized the photic and circadian expression of fosB as well as c-fos, c-jun and junB by in situ hybridization in hamsters. fosB mRNA exhibits a typical IEG kinetic of induction in response to a pulse (30 min) of light (during the dark phase of an LD 14:10 schedule) in cells of SCN, and mRNA levels are elevated for up to 150 min after the light pulse. When we assessed the spontaneous and light-evoked expression of these genes at different circadian phases, we found light-induced expression for all these genes during the subjective night, but c-fos, and especially junB also showed increased expression either spontaneously or after a light pulse at the beginning of the subjective day. These results suggest that induction of some IEGs in the SCN occurs spontaneously as well as in response to light stimuli at selected circadian phases. (Supported by CONICET of Argentina and the MRC of Canada).
CALMODULIN-DEPENDENT PROTEIN KINASE II EXPRESSION DURING WAKEFULNESS IN THE HIPPOCAMPUS OF BONED BATS: A GEMINI APPROACH TO REGULATORY MECHANISMS OF CALCIUM LAUNCHES. J. S. T. Ellis and A. J. Ziegler.}

There is strong evidence that wakefulness (W) and sleep (S) are associated with changes in the expression of certain genes, including CaMK-II, which is often limited during the period of sleep. The loss of dye from the cell and its gradual compartmentalization into regions that are not representative of cytosolic Ca2+. Dendritic-conjugates of Ca2+ are present in the wakefulness state, but their usefulness with small neurons such as those of the suprachiasmatic nucleus (SCN). Because the SCN is the principal mammalian circadian pacemaker we wished to monitor CaMK-II in SCN cells for at least 3 days. Such a capability would provide [Ca2+] measurements in individual SCN cells throughout the circadian cycle and would allow the effects of phase-shifting agents (e.g., cold) to be measured. For long-term Ca2+ imaging, primary cell cultures of neonatal rat SCN were loaded with the dye Fura-PE3 AM, a modified form of the membrane-permeable Fura-2 AM which is reported to show less compartmentalization and leakage than Fura-2. Cells were loaded for 1 to 2 hours with 10 μM Fura-PE3 AM and then left at room temperature (22°C) in a HEPES-buffered medium. The emission at 510 nm was imaged during alternating 340 and 360 nm light pulses. The cells showed a diffuse cytoplasmic fluorescence for at least 50 hours after loading and elevated K+ treatments caused reversible (Ca2+) increases in fluorescent intensity during this time. This approach can be used to measure [Ca2+] in SCN cells during the circadian cycle. Supported by NIH NS12864 and the NSF Center for Biological Timing.

658.22\textbf{CONSTANT LIGHT HUSO INDUCES FOS PROTEIN IN RAT INTERGENICULATE LEAFLET.} E. Edelstein and S. A. Amir, Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

Exposure to prolonged constant light (LL) disrupts circadian rhythms in neuronal rodents. This disruption may be manifested as a loss of circadian rhythmicity, desynchrony of individual rhythms, or splitting of circadian activity rhythms into two or more components. The neural mechanisms underlying these effects is not well understood, although previous research has demonstrated that ablation of the intergeniculate leaflet (IGL) prevents splitting of circadian activity rhythms in the hamster. We examined the effects of LL on the circadian system of Wistar rats previously housed under a normal light-dark cycle, using a telemetry system to measure temperature and activity rhythms, and the interrelations between tempeature and activity. We found that fos expression was observed in the IGL of those animals. Whereas Fos expression was observed in the IGL of rats sacrificed during the LL period prior to the loss of circadian rhythmicity, rats sacrificed after this disruption occurred tended to exhibit fewer fos immunoreactive cells in this region. Results indicate the idea that the IGL mediates the disruption of circadian rhythms during exposure to prolonged LL. These data also suggest that Fos expression in the IGL may be a marker of the integrity of the circadian system in rats housed under prolonged LL.

658.24\textbf{CIRCADIAN CHANGE IN THE INTENSITY OF ULTRaweak BIOCHEMILUMINESCENCE FROM RAT SUPRACHIASMATIC NUCLEUS SLICE.} Y. Ito, Y. Itoh, T. T. Nakagawa, N. Naka, K. Hikida, and H. Nakagawa. 1 Division of Protein Metabolism, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan. 2 Photodynamics Research Center, The Institute of Physical and Chemical Research (RIKEN), Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN). Faculty of Engineering, University of Tokyo.

UCS Classed light-emitting diodes (LEDs) are related to biological phenomena are known. One is the well-known bioluminescence visible to human eyes which is associated with reactions such as the luciferin-luciferase system. The other is ultraweak biochemiluminescence in the order of 10−16 W/m² which is spontaneously emitted from all kinds of living organisms without excitation light. In the previous work, we developed ultraweak sensitivity photodetection system using a silicon avalanche photodiode and scintillation detector ultrasensitive biochemiluminescence from rat hippocampal slice. It was shown that the intensity of biochemiluminescence is at the order of 10−16 W/m² and related with neural activity. In the present experiment, we tried to study the occurence mechanism of circadian clock in the suprachiasmatic nucleus (SCN), we applied this photodetection system to the SCN slice culture and succeeded to detect ultraweak biochemiluminescence for up to 72 hours. The intensity of biochemiluminescence showed circadian change, though its pattern was quite different from electrical or metabolic activity of the SCN. The intensity showed peaks in the middle of subjective night as well as subjective day, and troughs in the transition periods of the day and night. This result suggests that ultraweak biochemiluminescence from the SCN reflects not only neural activity but also other cellular activity.
658.25

VARIous TYPES OF SLEEP DEPRIVATION HAVE DIFFERENT EFFECTS ON CEREBRAL PROTEIN SYNTHESIS IN THE RAT. P. Ramul and R.K. Zohbski.
Dept of Psychology, Brock University, St. Catharines, ON L2S 3A1, Canada.
We investigate possible restorative functions of sleep by mapping cerebral protein synthesis (CPS) during sleep wake states. In this study, we compared CPS during post-deprivation recovery sleep under three conditions: a) short period of manual total sleep deprivation; 24 hr of island platform mixed sleep deprivation, and computer-controlled total sleep deprivation. Rat electroencephalograms for recording of EEG and EMG, and cannula allowing external access to the femoral artery and vein. A 3 day period was then allowed for recovery from surgery. Control animals (n=16) were also prevented from entering sleep for 4-6 hr. This condition represents minimal deprivation and a uniform high level of sleep need. A 24 hr platform condition (n=11) used medium-sized islands over which the condition replicates classic REM sleep deprivation. A final deprivation condition (n=9) used 24 hr in a computer-driven rocking cage, which was produced total sleep deprivation (>99%). Following the deprivation period, the animals were allowed to sleep. L-[14C]linoleic acid was injected, tissues collected, and tissue samples were taken for LSC and analyses of free amino acids, and the EEG/EMG were recorded for state monitoring. After 45 min, the brains were extracted, cut in a cryostat, and processed for quantitative autoradiographic analysis of CPS.

In the minimal deprivation (tired) and platform (some SWD deprivation, total REM deprivation) conditions, we observed a recovery phase between recovery SWD and rates of CPS. In contrast, the more deprived group (total sleep deprivation) exhibited a strong (p < 0.01) negative relation between CPS and SWD. These data suggest that the metabolic consequences of classic REM sleep deprivation are different from those of total sleep deprivation.

658.26

RECOVERY SLEEP IN Fos-LacZ mice leads to a rapid decline of the elevated Fos and 8-gal levels which result from forced waking. J.E. Shera, P. Shimomura, Morgan and C.B. Saper, Harvard Med. School, Boston MA 02115 & Rhode Insr. of Molecular Biology, Nutley N.J. 07110.

Previously we reported that sleep-deprivation (SD) increases the level of Fos-protein in the brain of the rat cat brain. Rapid recovery sleep (RS) following SD leads to its rapid decline. Using transgenic Fos-LacZ mice (n=6), we examined the effects of SD and RS on Fos and 8-gal expression in brain. Mice were deprived of sleep for 3 hours. These mice were sacrificed for analysis immediately after deprivation while some mice were allowed to sleep or wake spontaneously for 1-0.15 and 2.0 hours, at which point they were sacrificed for analysis.

Brains from animals not allowed recovery showed elevated high levels of 8-gal and Fos which were particularly prominent in cingulate and piriform cortices. In contrast, brains from animals which sleep spontaneously following deprivation showed a dramatic decrease in Fos and Fos.

These preliminary results in Fos-LacZ mice corroborate findings in rat and cat that Fos and now 8-gal protein degradation is enhanced during sleep. These results increase in degradative processes at the cellular level during sleep may be central to the functions of sleep. Thus, the efficient elimination of gene products that accumulate during waking may serve a restorative function at the cellular and ultimately the organismal level.

INVESTIGATIVE LEARNING AND BEHAVIOR V

659.1


The phosphorylation state of cp20, a low-MW membrane-associating GTP-binding protein, was previously shown to increase 2- to 3-fold in *Hermisenda* 24h after associative conditioning. Iontophoretic injection of either cp20 or protein kinase C (PKC) reproduces the biophysical and structural modifications that accompany memory acquisition. Associative conditioning causes translocation of PKC from the cytosol to membrane. Here, cp20 is shown to be phosphorylated by PKC. Marked differences in activity were observed with the three major isoforms of PKC, while casein kinase, CaM-kinase II, and cyclic AMP-dependent protein kinase produced no detectable phosphorylation of cp20, suggesting that the increase in phosphorylation of cp20 after conditioning is due to PKC. Phosphorylation of cp20 had no effect on its GTPase or GTP binding activity, but caused a translocation of cp20 from cytosol to the nucleus / mitochondrial fraction.

659.2


*Hermisenda* recent evidence suggests that facilitation at type B to A cell synapses may also contribute to learning in this system. Bonnem (G-1) has been implicated in both type B cell excitability and synaptic strength changes, and -amino butyric acid (GABA) has more recently been linked to type B cell excitability changes. Here we examined whether GABA also promotes facilitation at type B to A cell synapses. Rapid bath perfusion with 100 nM GABA followed by a seawater rinse (*GABA + RINSE*) enhanced the magnitude of inhibitory post synaptic potentials (IPSPs) elicited by type A cells by type B cell stimulation (mean change ± SEM: +0.90 ± 0.18 mV; n=2, p < 0.001.) In both preparations, control IPSPs were preceded by a large IPSP (0.3 ± 0.18 mV; n=18, 85% of 100 μM GABA (0.24 ± 0.21 mV; n=11, NS) showed no significant changes. An ANOVA (F=11.86, p < 0.001) followed by Duncan pairwise comparisons revealed that the changes in IPSP amplitude observed in the GABA + RINSE and 5-HT conditions were both significantly greater than the changes in the other two conditions (p<0.1), which did not differ. The observation that *GABA + RINSE*, but not sustained GABA exposure, promotes synaptic facilitation suggests that when GABA is present, its slow, modulatory effects on synaptic strength may be counteracted by its faster shunting actions; hence, removal of GABA from the bathing medium may have revealed its more sustained modulatory actions. Because the GABA + RINSE condition approximates the transient US-mediated activation of GABAergic hair cells which occurs during associative training of the intact animal, these findings suggest a straightforward means by which behavioral training could promote synaptic facilitation in this system.

659.3


Recent reports suggest that the LE sensory neurons may not be activated by a light touch that initiates a gill-withdrawal reflex. We have used voltage-sensitive dye recordings to evaluate the role of LE sensory neurons in the gill-withdrawal reflex. In a preparation of a ganglion in sea water, we detected action potentials in about 100 neurons in response to a 1 or 2 g touch. The gill withdrawal in response to this stimulus was vigorous and often seemed to be more than half as large as the response to a 5 g touch. Additional recordings were made in a low calcium or a high divalent sea water. This suppressed the activity of interneurons and motor neurons but presumably did not affect sensory neurons. In experiments on five animals we estimated the number of cells meeting LE-cell criteria (i.e., small, no spontaneous activity, activated by touch in altered divalent sea water, located in the LE region). In response to a light touch a median of 5 cells (range 0 to 8) fired a median of 1.8 spikes/cell (range 0 to 2.9). Given the small spike size in gill motor neurons from LE spikes relative to the overall PSp size in responses to siphon touch (Bryne et al., J.Neurophysiol., 1978), this low activity level is consistent with the existence of other sensory cells responsive to light touch. We estimate that the LE contribution to the motoneuron PSp for light to moderate touch ranges from 0 to 10% depending on the strength of the touch and the behavioral state of the preparation. We are presently recording from other regions of the abdominal ganglion and from other ganglia to search for cells briskly activated by siphon touch in altered divalent sea water. Supported by NSF grant IBN-9222214, and NIH grants NS08437 and NS07102.

659.4

NOCICEPTIVE RESPONSES AND SENSITIZATION OF LE SIPHON SENSORY NEURONS IN *APLYSIA*. D.A. Illich* and E.T. Walters. Dept. of Integrative Biology, Univ. Texas - Houston Medical School, TX 77000.

Responses of LE sensory cells to mechanical stimulation were compared in 1) a semi-intact preparation that allows unrestricted siphon movements and 2) a pinned out preparation like that originally used to characterize the LE cell response properties (Bryne, Castellucci & Kandel, J. Neurophysiol. 39:407-414, 1978). In both preparations the LE cells showed maximal activation (including afterdischarge for up to 15 sec) by strong siphon pinch. LE cells displayed a very wide dynamic range to von Frey hair stimuli, sometimes responding weakly to innocuous pressure, and showing graded activation to increasing pressures. Significant depression of mechanosensory threshold was produced by either pinching the unrestrained siphon or by testing the siphon in the pinned out preparation. Siphon pinch or siphon pinning also dramatically increased some excitability. Water jets that activated LE cells in the pinned out preparation rapidly evoked responses in the unrestrained siphon. Weak vibratory stimulus applied several cm away from the siphon never activated LE cells, while reliably activating a set of unidentified neurons in the abdominal ganglion. These include 1) that LE cells can encode nociceptive information, as well as information about innocuous mechanical stimuli, 2) that receptive field injury greatly enhances peripheral and central excitability of the LE cells, and 3) in an extension of previous observations (see Cohen et al. Soc. Neurosci. Abstr. 17:1302, 1991), that *Aplysia* has unidentified siphon mechanoreceptors that are far more sensitive to weak mechanical stimuli than the LE cells are.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
569.5 OCTOPAMINE MAKES LOCUSTS PAY ATTENTION
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Like schoolchildren, insect experience boredom, showing declining responses to repetitive visual patterns. However, both can be curbed by suddenly presenting an additional, novel stimulus. A clear example of arousal in the locust is provided by the visual system: the habituated response of the descending ganglion served by the second (DNM2) of the three locust brain optic lobes may be habituated (DLM2) by a novel stimulus (Pena et al., 1994). We believe octopamine mediates arousal in this system because its exogenous application to the locust brain and optic lobes can displace the DLM2 much like tactile stimuli do. The locust CNS contains 4 octopamine-immunoreactive neurons, the protocerebral medulla (P6454) neurons, that could potentially mediate this effect. P6454 neuron projects from the deutocerebral protocerebral lobes, and into the optic lobe. Map specificity has been confirmed that each P6454 cell body contains approximately 25,000 octopamine. Activity in P6454 neurons is increased by tactile stimulation of the locust head or body, by auditory stimuli or by light or other visual stimuli. To provide categorical evidence that P6454 neurons can habituate the DLM2, we recorded extracellularly from the DLM2 and intracellularly from one of the P6454 neurons. When P6454 action potentials were suppressed with hyperpolarizing current, the DLM2 habituated to a moving visual stimulus. However, depolarizing a P6454 neuron, to produce action potentials at approximately 200Hz, significantly increased the number of DLM2 action potentials per stimulus. The P6454 neuron therefore plays an important role, presumably by the release of endogenous octopamine within the optic lobe, in dishabituation of DLM2 by novel stimuli.

569.6 CELLULAR ANALYSIS OF HABITUATION OF THE PROLEG WALK-WITH REFLEX OF LARVAL MANUDA SEXTA. D. E. West, E. R. Wood and J. W. Weeks
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Stimulation of plant hairs on an abdominal proleg of larval Manduca sexta evokes withdrawal of the proleg toward the body wall. The proleg withdrawal reflex (PWR) habituates with repeated detection of an alert and attention or arousal by suddenly presenting an additional, novel stimulus. A clear example of arousal in the locust is provided by the visual system: the habituated response of the descending ganglion served by the second (DNM2) of the three locust brain optic lobes may be habituated (DLM2) by a novel stimulus (Pena et al., 1994). We believe octopamine mediates arousal in this system because its exogenous application to the locust brain and optic lobes can displace the DLM2 much like tactile stimuli do. The locust CNS contains 4 octopamine-immunoreactive neurons, the protocerebral medulla (P6454) neurons, that could potentially mediate this effect. P6454 neuron projects from the deutocerebral protocerebral lobes, and into the optic lobe. Map specificity has been confirmed that each P6454 cell body contains approximately 25,000 octopamine. Activity in P6454 neurons is increased by tactile stimulation of the locust head or body, by auditory stimuli or by light or other visual stimuli. To provide categorical evidence that P6454 neurons can habituate the DLM2, we recorded extracellularly from the DLM2 and intracellularly from one of the P6454 neurons. When P6454 action potentials were suppressed with hyperpolarizing current, the DLM2 habituated to a moving visual stimulus. However, depolarizing a P6454 neuron, to produce action potentials at approximately 200Hz, significantly increased the number of DLM2 action potentials per stimulus. The P6454 neuron therefore plays an important role, presumably by the release of endogenous octopamine within the optic lobe, in dishabituation of DLM2 by novel stimuli.

569.7 CHARACTERIZATION OF A MORPHologically DISTINCT SUBSET OF SENSORY NEURONS IN THE PLEURAL GALLONGANGLIA OF APHIS. H. Zhang, J. J. Byrne and J. L. Henahan
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The tail-siphon withdrawal reflex in Aphis is a useful model system for studying simple forms of learning and memory. The sensory neurons mediating the reflex are located in a cluster on the ventrolateral surface of the ganglion. Most of these neurons project to the central nervous system (CNS) to which they are connected to the head (Zhang et al., 1993). A small number projected through the cerebral-pleural connective. Because of their unusual projection, we wanted to confirm that these neurons were sensory in function. Members of this cluster (n=20) were identified by staining with immunoreactive cellular nervous tissue. Their location on the rostral edge of the cluster corresponded to the subset of sensory neurons labeled by backfilling the cerebral-pleural connective. Tactile stimulation of the head skin elicited action potentials without pretreatment. Hyperpolarization of the cell body reduced the amplitude of the action potentials. Most of these sensory neurons had receptive fields on the anterior tentacle (n=17), and a few had receptive fields on the posterior tentacle (n=2) and lips (n=1). Physiological-identified cells were injected iontophoretically with 2% dextrose stereotacticly to Hassler, and their receptive fields were located in the cerebral-pleural and pleural-pedal connective. Some of these neurons were bipolar (n=8). In 10 preparations, axons could be traced out cerebral nerves (C1+C2), C2+7, C4+2). In 2 preparations they passed through the cervical ganglion. Our results suggest that a subset of sensory neurons located on the rostral edge of the pleural sensory cluster are primary mechanoreceptors for the head. These neurons are morphologically distinct in that they are either bipolar or bifurcate within the pleural ganglion. Functionally, they may be more closely related to sensory neurons in the J and K clusters of the cerebral ganglion, which also project to the pedal and pleural ganglia, than to other sensory neurons in the pleural cluster.

569.8 CELLULAR CORRELATES OF LONG-TERM SENSITIZATION IN APLYSIA. W. I. Weast, M. Aguirre, J. L. Henahan, J. J. Byrne
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Long-term sensitization is an important form of learning exhibited by the tail-siphon withdrawal reflex. Previous work demonstrated that this behavioral effect was lateralized, and was correlated with changes in the membrane properties of tail motor neurons (Scholtz et al., 1987). In this study, we extended these observations to include properties of both sensory and motor neurons innervating the tail, as well as the synaptic connections between them. Animals were trained as described previously (Scholtz and Byrne, 1987). Enhancement of siphon withdrawal was greater on the sensitized side of the animal than on the contralateral control side (184 ± 146% vs. 121 ± 7%, P < 0.001). Training increased the excitability of the contralateral sensory neurons from the sensitized side of the animal. A depolarizing current pulse (2 nA, 1 sec) elicited 1.4 ± 0.1 spikes on the sensitized side compared with 0.4 ± 0.1 spikes on the contralateral side. Moreover, the afterdepolarization following the pulse was enhanced in sensitized ganglia compared with controls (7.7 ± 0.8 mV vs. 0.7 ± 0.3 mV, P = 0.03). Resting membrane potential and input resistance were unchanged. Training had no significant effects on properties of tail motor neurons, including resting membrane potential, input resistance and spike threshold. Synaptic currents elicited by the action potential in a sensory neuron were calculated by dividing the amplitude of the evoked input by the input resistance of the motor neuron. Training enhanced the calculated synaptic current (1.85 ± 0.33 nA vs. 0.92 ± 0.33 nA, P < 0.05).

In these experiments, long-term behavioral training produced a lateralized enhancement of sensory neuron excitability, which is consistent with the change in net outward currents described previously (Scholtz and Byrne, 1987). Synaptic transmission between sensory and motor neurons mediating tail withdrawal was also enhanced in the trained side of the animal. Therefore, these experiments confirm that the same cellular changes induced in vitro (Zhang et al., 1994) are correlated with long-term sensitization in vivo.

569.9 CHANGES IN CALMODULIN (CAM) AND ROLE OF CAM-DEPENDENT PROTEIN KINASE II IN SYNAPTIC PLASTICITY IN APLYSIA. F. Luck, M. P. V. O'Sullivan, K. Nakashima, D. A. Baxter, S. Hart, O. R. Liu, K. Maggert, A. Eskin, and J. J. Byrne
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Experiments using in vivo translation of mRNA indicated that the level of mRNA for calmodulin (CAM) was increased in vivo in analogue of sensitization training in Aplysia (Zawartki et al., 1992). This raised the possibility that CAM and Ca2+calmodulin-dependent protein kinase II (CaMKII) may play a role in the induction of the long-term sensitization (LTS) and long-term facilitation (LTF) and LTD. Indeed, we previously found that KN62, an inhibitor of CaMKII, reduced LTS-induced STP and LTD-induced potentials in the pleural-pedal ganglia (Nakashima et al., 1994). The present studies have extended these findings. First, using ribosome-protein kinase assays, we found that CAM and CaMKII are increased in sensory neurons (SN) induced by 5-HT, and also by behavioral training, which induced long-term sensitization (see Lee et al., this volume). Second, we examined LTF in isolated pleural-pedal ganglia. The amplitudes of EPSPS at identified sensory connections were measured before and 24 hr after nerve stimulation, an analogue of long-term sensitization training. Neither KN62 nor KN94 (inactive form of KN62), applied during stimulation, blocked the induction of LTF. Third, to test the possible role of CAM in the maintenance of LTF, we continued the experiment by applying KN62 and transported EPSPS again 15 min later. KN62 did not block the expression of LTF. Finally, we examined the effects of KN62 on 5-HT-induced spike broadening and increases in excitability in SN. KN62 was applied to isolated SN neurons after baseline tests (3 min) and 1 min after the application of 5-HT. KN62 by itself induced a slight increase in the duration of the action potentials, but did not block the 5-HT-induced excitability. In addition, KN62 had no effect on either basal excitability or the 5-HT-induced increases in excitability. These data suggest that a spike duration independent (SDI) process contributes to STF and that CaMKII plays a role in this SDI process. Although CaMKII does not appear to be necessary for LTF, calmodulin may act through mechanisms other than CaMKII.
659.11

Long-term memory in Aplysia requires protein and RNA synthesis and can be induced by exposing neural tissue to serotonin (5-HT) (Montelongo, 1986). Using in vitro translation of mRNA and two-dimensional polyacrylamide gel electrophoresis of the protein products, we previously found three proteins, calmodulin, phospholipase A2 and an unidentified one (protein 3) whose mRNA levels were increased in response to treatments that mimicked sensitization training (Zwartwout et al., 1992). To identify protein 3, we screened a Lambda Zap library of Aplysia ganglia with a 132 bp PCR probe amplified using degenerate oligonucleotides designed from the peptide sequence. A 1750 bp cDNA clone controllable by the DNA sequence that codes for the original peptide (58 amino acids), was isolated. An amino acid sequence of 210 residues deduced from the middle of the cDNA clone of protein 3 yielded no similar sequences in a search of Genbank. Approximately 90% of the cDNA clone has been sequenced in one direction. We are currently sequencing the opposite strand. To confirm the change in levels of mRNA we initially observed, ribonuclease protection assays have been used to measure mRNAs. Levels of protein 3 mRNA in pleural sensory neurons were increased by 383%±0, n=5, when ganglia were exposed to 5-HT for 1.5h. Moreover, long-term behavioral training also increased protein 3 mRNA (48±11%, n=6) in sensory neurons.

659.13
LONG-TERM HYPERSENSITIVITY OF APLYSIA SENSORY NEURONS: INTERACTIONS OF ATOXOMY AND 5-HT. X. Liao, J.D. Gunstream*, T. Morgen, and E.J. Walters*. Department of Integrative Biology, Univ. of Texas - Houston Medical School, 77003.

Both repeated 5-HT application to sensory neurons (SNs) in dissociated cell culture (Dale & Kandel, J. Neurosci. 7:252, 1987) and close anatomy of SNs in organ culture (Gunstream et al., J. Neurosci. 15:439, 1995) enhance soma excitability 1 day later. To begin to explore potential interactions between signal pathways activated by axon injury and by 5-HT, we have examined the effects of 5 applications of 5-10 μM 5-HT on SNs axotomized different distances from the soma 1-2 hours earlier. 5-HT treatment enhanced the excitability (tested 20-24 hrs) of 50% of the later) of SNs whose axons had been crushed 10-20 mm from the soma (8.6±0.7 spikes after 5-HT vs. 6.0 ± 1.1 spikes in controlateral, p<0.02; n=7 animals). This distance is short enough to allow retrograde transport of axonal injury signals to the soma within 20 hr. In contrast, 5-HT application had little effect on the excitability of SNs in which the distance to the axonal crush was too long (>40 mm) for such signals to reach the soma by this time (4.5 ± 1.2 vs. 4.2 ± 0.7 spikes, n=5). [However, 1 of these 5 "long nerve" preparations did show a significant within-animal effect of 5-HT.] Repeated application of 50 μM 5-HT had no obvious effect in the "long nerve" preparation (3.5 ± 3.5 spikes, n=2 animals). These results suggest that axonal injury signals may permit 5-HT-induced long-term hypersensitivity to be expressed. Injury signals produced by extremely close axotomy (excluding the SN soma) were not sufficient to permit 5-HT-dependent soma hypersensitivity (0.9 ± 1.3 after 5-HT vs. 6.8 ± 0.7 spikes, n=5 animals), indicating that interactions between 5-HT and axotomy signals are complex and depend upon the site of axonal injury.

659.14
ACETYLCHOLINE INDUCED HYPERSENSITIVITY OF APLYSIA SENSORY NEURONS REQUIRES PERIPHERAL CALCIUM. J.D. Gunstream*, G.A. Castro and E.J. Walters. Department of Integrative Biology, Univ. Texas - Houston Medical School, 77003.

Nerve injury in Aplysia leads to long-term hypersensitivity of the central somata of sensory neurons (SNs), which is triggered (at least in part) by retrograde transport of axonal injury signals to the soma (Gunstream et al., J. Neurosci. 15:439, 1995). To begin to define initial cellular signals of axonal injury we used an in vitro preparation in which peripheral nerves containing axons of VC SNs could be treated selectively. 24-30 hr after proximal nerve crush, a 1 sec depolarizing pulse at 2.5x threshold elicited 6.8 ± 0.8 spikes in axotomized SNs, whereas paired, contralateral SNs whose axons were left in 0 Ca²⁺ (normally) or 0 Ca²⁺ and EGTA displayed only 3.8 ± 0.3 spikes (p<0.001, n=29 cell pairs). A solution containing 100 μM Ca²⁺ did not prevent hypersensitivity (7 spikes vs. 6.5 spikes in normal Ca²⁺, n=8 cell pairs). Elevation of Ca²⁺ alone does not appear sufficient to trigger hypersensitivity since calcium ionophores (100 μM ionomycin or calcium ion) applied to long nerves (which do not exhibit hypersensitivity within 30 hr) had no significant effect (4.0 spikes vs. 4.4 spikes without ionophores, n=24 cell pairs). However, an initial study using 100 μM BAPTA-AM (which is membrane permeant) suggests that an elevation of intracellular Ca²⁺ may be necessary for triggering long-term hypersensitivity. How Ca²⁺ exerts its necessary role(s) is not known. Preliminary experiments have revealed no consistent effects on hypersensitivity of bathing the nerve crush site with several nonspecific inhibitors protein kinases (K-252a, staurosporine, and KN-62; 5-20 μM each).

659.12

Olfactory stimulation of antennal receptors can release the cardiac response of premature heart-beat reversal in the blowfly. Repeated stimulation with 1-hexanol vapors can iteratively evoke the response or lead to response habituation depending on the efficacy of the chemical concentration in inducing behavioral responses of rejection as well.

By simultaneously recording heart activity and sensory input on intact flies, we examined the dynamics of acquisition and retention of response habituation as a function of stimulus intensity and interstimulus interval (ISI). The response habituates faster to low intensity stimulation repeated at the shortest and longest ISI. Duration of habituation retention is directly related to stimulus intensities adopted, and it ranges between 30 and 120 mean values. The response to olfactory stimulation is promptly restored in habituated flies, i.e. dishabitation takes place, following intense stimulation of mechano-taste receptors on the labellar area.
660.1

Conditioned flavour aversions (CFAs) can be produced by pairing ingestion of a novel food with exposure to a toxin, such as LiCl. Using a rapid, within session, conditioning paradigm, the effects of pairing a novel taste (saccharin), odor (almond), or taste-odor mixture (saccharin-almond) with LiCl treatment, on conditioned changes in taste reactivity responses (TRRs) were examined. On 2 occasions male rats were injected with NaCl or LiCl (3 meq/kg, i.p.) placed in an observation box for 30 min, and given 7 brief (10 sec) intraoral infusions (0.78 ml/min) of a sodium saccharin (0.2%), almond odor (2%), or saccharin-almond mixture dissolved in distilled water, at 5 min intervals. Relative to the NaCl control rats, the LiCl treated subjects exhibited significant (p<0.01) conditioned reductions in ingestive TRRs and increased levels of passive drip. When tested with saccharin or almond alone in a CFA test phase (no injections), conditioning to the taste or odor cue was found to be equally strong. The present results provided no evidence for a taste-odor conditioned odor conditioning effect. (Supported by NSERC and MRC of Canada).

660.3
AMYGDALA LESIONS ATTENUATE PICA ELICITED BY LITHIUM CHLORIDE INJECTIONS BUT NOT BY FOOD DEPRIVATION IN RATS. D. Machulak, J. Harlan and B. Tarpley. Dept. of Psychol., Univ. of Calif., Los Angeles, CA 90089, Dept. of Psychol., Univ. of Calif., Los Angeles, CA 90004.

The amygdala has been shown to play a role in a wide spectrum of visceral phenomena including gastric motility, secretion, pathology, and gastrointestinal malaise. In previous research, we have demonstrated that pica, the consumption of non-nutritive substances, is a useful animal model for quantifying unpleasant visceral sensations produced by a variety of treatments such as food deprivation and lithium chloride injections. We have conducted two experiments investigating the effects of bilateral electrolytic (Experiment 1) and isotonic (Experiment 2) amygdala lesions on food deprivation- and illness-elicitated pica. In Experiment 1, lesioned and sham-operated rats were given food and water, and kept on a non-nutritive diet (non-nutritive diet) continuously available throughout the testing sequence, except for the initial food deprivation test during which the food was removed from all rats for 24 h. Two weeks following surgery, the rats were tested three days following the food deprivation test, half of the sham rats and rats with amygdala lesions were administered lithium chloride (0.15 M, 20 ml/kg, i.p.) every third day for a total of five treatments. The remaining sham rats were treated identically except that they were administered an equivalent volume of isotonic saline (0.15 M) on each of the five treatment days. Though all three groups engaged in comparable amounts of deprived-elicited pica, amygdala lesions eliminated the robust increase in illness-elicted pica apparent in sham given repeated lithium chloride treatments. Saline controls did not engage in pica on any treatment day. A similar pattern of results was obtained in Experiment 2 with isotonic saline lesions in the amygdala. These results reveal that neurons in the amygdala mediate behavioral responses to the visceral malaise induced by lithium chloride, but not by food deprivation, and they further demonstrate the utility of pica as an effective tool in studying the psychophysics of subjective visceral sensation.

660.5

The induction of c-fos-like immunoreactivity (c-FLO) in the intermediate nucleus of the solitary tract (INTS) is a neuronal correlate of conditioned taste aversion (CTA) expression (Hopt et al., 1994; Swank et al., 1994). Decrease preparations have demonstrated that the expression of Fos-like immunoreactivity to the hindbrain and forebrain are required for behavioral expression of a CTA. The CaNCe is a candidate forebrain site required for CTA expression. In this study, we examined the role of c-FLO induction and CTA expression in the CenNCe as a marker of neuronal activation during CTA expression. Adult male rats were implanted with sublabial intracranial catheters. Conditioned rats received intraoral infusions of 5% sucrose (3ml/min) paired with a LiCl injection (12ml/kg, 0.15M ip) 1 times over 1 week. Unconditioned controls received LiCl and sucrose on alternate days (non-consecutive) to the CTA to assess sucrose were acquired. Two days after the last pairing rats received an intraoral infusion of 5% sucrose (3ml/min). One day later, rats were sacrificed and processed for c-FLO. Positive cells were counted within a 0.23 mm radius centered on the CeNCe in the conditionNCe and unconditionNCe rats. An intraoral infusion of sucrose induced more c-FLO-positive cells in the CenNCe of conditioned rats (68.5 ± 15.1, n=3) than a sucrose infusion did in unconditionNCe rats (21.2 ± 2.6, n=3). An acute LiCl injection (0.15M, 12ml/kg) also induced large numbers of c-FLO-positive neurons in the CenNCe (155.0 ± 9.7, n=3). All 3 groups were infused with saline. Thus the CeNCe responds differently to an intraoral sucrose infusion at the level of gene expression after acquisition of a CTA against sucrose. The observation of altered c-FLO induction in the CeNCe complements and parallels our observation of altered c-FLO induction in the INTS. The CeNCe has reciprocal connections with many brain regions, including the medial INTS. This result shows the possibility that the induction of c-FLO in the INTS during CTA expression is causally linked to activation of the CeNCe. Supported by the Whitfield Fdn and NY Obesity Cr (TAH) and MHR0149 (GPS).

660.6
EXCITOTOXIC LESIONS OF THE RAT LATERAL HYPOTHALAMUS: EFFECTS ON TASTE AVERSION. J.M. Scollo, M.P. Latimer and P. Wint (OPEN, Brain Research Association) School of Psychology, Univ. St Andrews, Fife, Scotland KY16 9JU.

Excitotoxic lesions of the lateral hypothalamus (LiH) produce small reductions in intake; no motor impairments. Combined with additional offer of glucose or salt to the diet, normal responding to food and water deprivation (including prandial drinking). LiH lesions rats also show normal motivation measured by responding for food on progressive ratio schedules. There are though already impurities in responding to physical challenges such as hypertonic saline. Excitotoxic lesioned LiH rats also respond as controls to adulteration of the diet with sucrose or quinine, indicating normal sucrose processing. Taste aversion conditioning (the association of a specific taste with experimentally produced malaise) has not been investigated. To examine this, bilateral LiH (2.0 µl 0.9% NaCl in a phosphate buffer and control (2.0 µl phosphate buffer) rats were water deprived for 1day/3 days. On the fourth day rats were given 0.15% saccharin solution instead of water for 30 min. Intake did not differ between lesioned and control rats. Immediately after, half the LH and half the controls were given 20ml/kg of 0.15M LiCl: the remainder had an equal volume of NaCl. Behavior was observed for 30 min post-injection. All LiH treated rats showed a characteristic "taste-aversion" pattern, 3 days after this there was a two-bottle drinking test: one bottle contained 0.15% saccharin solution, the other tap water. Intake of saccharin solution was suppressed in both LH and control rats which had previously had LiH injections; injection of NaCl had no effect. In further tests, drinking in response to hypertonic saline was significantly suppressed in LH rats compared to controls. These data indicate that LH lesioned rats which characteristically fail to respond as controls to injections of hypertonic saline perform normally in tests of taste aversion.

660.4
THE EFFECT OF INSULAR CORTEX LESIONS ON LITHIUM CHLORIDE INDUCED BEHAVIORAL RESPONSES AND CONDITIONED TASTE AVERSION. P.A. Bryant, M.A. Norris and J. McGovern. Psychology, University of Sydney, NSW, 2006, Australia. Lithium chloride (LiCl) produces a range of behavioral and visceral phenomena in rodents and in males humans and is frequently used to induce conditioned taste aversions (CTA) in animals. In rats, LiCl induces a series of unconditioned responses including reduced food and water consumption as well as inducing nausea and emesis. In this study following LiCl injection and following exposure to conditioned and unconditioned taste stimuli. Water-deprived IC lesioned and sham lesioned rats were given 30ml access to 0.1% saccharin solution and then immediately injected (i.p.) with LiCl (0.15M, 20 mg/kg). They were assessed for unconditioned effects of LiCl (hypothermia, anorexia and LiOBl) for one hour. Starting two days later, they were given four daily one bottle test sessions: one bottle contained 0.1% LiCl and the other 0.1% saccharin was measured. IC lesions had no effect on any of the unconditioned responses to LiCl, with sham and lesioned animals exhibiting a similar level of LiOBl. Anorexia and hypertension. IC lesions also had no effect on neophobia to the 0.1% saccharin. However, IC lesioned animals showed significantly faster extinction of a CTA to saccharin.

This suggests that although the insular cortex is clearly activated by LiCl (as shown by c-fos expression) it does not mediate the unconditioned effects of LiCl. Rather it may be that the insular cortex is involved in the associative mechanism whereby taste and illness are paired.

660.8
VARIATION IN AVOIDANCE OF BITTER COMPPOUNDs BY RODENTS AND BIRDS. S.A. Wagner-Parlab and J. R. Mason. USDA/APHIS/ADWR/ c/o Monell Chemical Senses Center, Philadelphia, PA 19104.

Quinine hydrochloride (QH) and denatonium benzoate (DB) are non-toxic, chemically dissimilar, bitter tasting compounds. The perceived toxicity of bitter compounds can modify dietary selection by rodents and birds. We evaluated responses of two rodent species, deer mice, Peromyscus maniculatus and mice, Microtus montopus, and an avian species, European starlings, Sturnus vulgaris to the bitter taste of QH and DB. The effect of adulteration of a preferred food, apples, by soaking in QH (0% 0.1% and 0.5% v/v) and DB solutions was evaluated during 2 hr, 2 choice feeding trials. Both DB and QH (0.01 and 0.1% v/v) decreased apple intake in mice, voles, and starlings P<0.05. Voles exhibited greater avoidance of QH (0.1%) than DB (0.01%) selecting 27.8% of their intake from the QH adulterated apples compared to 39.8% from DB treated apple pieces in separate trials, P<0.05. Voles avoided QH at 0.1% and DB at 0.01% intake. European starlings were less sensitive than either deer mice or prairie voles to these bitter substances requiring higher concentrations of QH and DB to inhibit intake. Despite intraspecies variation in responsiveness, both rodents and birds avoided QH and DB adulterated apples. Therefore, these compounds may be useful in wildlife management of a diverse range of species. (This work was supported by a cooperative agreement between USDA/APHIS/ADWR and Monell Chemical Senses Center.)
660.7 FOREBRAIN CONTRIBUTION TO THE CONDITIONED EXPRESSION OF C-FOS IN NTS FOLLOWING TASTE AVERTION LEARNING. Glenn E. Sachee, Randy J. Seselaj, & Ilene L. Bernstein*. Department of Psychology, University of Washington, Seattle, WA 98195

The induction of c-fos-like immunoreactivity (c-FLI) in intermediate NTS following re-exposure to a taste (CS) which has been paired with LiCl appears reliable correlate of the behavioral expression of a conditioned taste aversion (CTA). This pattern of c-FLI is similar to that seen following administration of the US drug (LiCl). The present studies employed a variant of the chronic de cerebrate rat preparation to explore whether circuitry intrinsic to the brainstem is sufficient to support the induction of c-FLI in NTS following both administration of the US drug and re-exposure to the CS taste. In the first experiment, chronic hemispherectomy rats, which have a unilateral supracarotid brain transection, were injected with either LiCl or NaCl. Hemispherectomies in the second study were re-exposed to a saccharin CS which had been either paired or unpaired with LiCl. Results indicated that the bilateral induction of c-FLI following LiCl administration was unaltered by the transection, while expression of c-FLI following exposure to the CS taste was evident only on the side of the brain which retained neural connections with the forebrain. These findings indicate that forebrain connections are necessary for the induction of c-FLI in NTS during the behavioral expression of a CTA, but not for that following administration of LiCl. Thus, in the CTA paradigm, two distinct neural pathways appear to mediate the response to the US (LiCl) and CS (taste).

660.8 ROLE OF THE AREA POSTREMA IN CISPLATIN-INDUCED TASTE AVERTION LEARNING IN RATS. B. M. Rubin*, Dept. of Psychology, Univ. of Maryland, Baltimore, MD 21201, U.S.A.

A conditioned taste aversion (CTA) is produced when ingestion of a novel food is paired with a toxin, such that the organism avoids ingestion of the food at a subsequent presentation. CTA learning following treatment with many toxins in dependent upon the integrity of the area postrema (AP), the chemoreceptor trigger zone for emesis. Two sets of experiments examined the role of the AP in the acquisition of a cisplatin-induced CTA and its relationship to a CTA produced by lithium chloride (LiCl).

Injection of cisplatin (3-5 mg/kg, ip) produced a significant test day reduction in the intake of the conditioned stimulus (10% sucrose solution) using a two-bottle test. However, interpretation of the findings was confounded by the observation that the injection of cisplatin also produced a reduction in total fluid intake. AP lesions prevented both the acquisition of the cisplatin-induced CTA and the reduction in fluid intake. Preexposing rats to 3 unpaired injections of LiCl (3 mg/kg, ip) prevented cisplatin-induced CTA learning. Preexposure also prevented the cisplatin-induced reduction in fluid intake.

The results support the hypothesis that the integrity of the AP is necessary for the formation of a cisplatin-induced CTA, and that the mechanisms by which cisplatin leads to the acquisition of a CTA are similar to those of LiCl.


Caloric intake may be largely determined by the palatability of food and many highly palatable foods are high in fat and high in sodium content. The present study was conducted to determine how taste and other oral responses to a high fat/high fat preload may account for overeating. The Three-Factor Eating Questionnaire was used to identify four groups of women according to history of dieting (High or Low Restraint) and tendency to binge eat (High or Low Vomiting). Half of the subjects were given a high sugar/high fat preload of chocolate pudding to eat and then were presented with a standard lunch. Those subjects in the Preload condition ate twice as many calories as did subjects in the control condition. Data will be presented to show how taste and other oral responses to the high sugar/high fat preload are associated with overeating in each of the four groups.


Retinopetal white adipose tissue (RWAT) and mesenteric white adipose tissue (MWAT) are more responsive than epididymal white adipose tissue (EWAT) to changes of food intake. Neural and/or humoral factors may be involved. To differentiate these possibilities, we changed the humoral environment by use of streptozotocin (STZ). Diabetic rats (STZ 80 mg/kg, glucose 400 mg/dl) were divided into two weight-matched groups and fed a high-fat diet (HF; n=15; fat = 43%, carb=19% by weight of diet) or a high-carbohydrate diet (HC; n=15; fat=6%, carb=70% by weight of diet). Non-diabetic controls (n=16) were fed 33% fat and 65% carbohydrate. Diabetics in the HC diet, but non-matched on the HF diet. Reproductive, body weights of HF (364 ± 8 g) and HC (345 ± 9 g) were not different. At sacrifice, 4 rats, body weights of HF (347 ± 12 g) and HC (345 ± 11) were not also different, nor were they different from beginning body weights. HC compared fat pad mean weight (5.0 ± 0.6 g) was reduced significantly (p<0.05) compared to HF combined fat pad mean weight (9.5 ± 0.8 g). HC had comparable EWAT (0.4 ± 0.3 g) and RWAT (0.7 ± 0.5 g) weights. However, HC had smaller RWAT (1.4 ± 0.3 g) than EWAT (2.2 ± 0.5 g). In controls, EWAT (4.4 ± 0.3 g) was heavier than EWAT (3.4 ± 0.2 g). Thus, in non-diabetic controls RWAT weights more than EWAT; in HF, RWAT weights the same as EWAT; and in HC, RWAT weights less than EWAT. MWAT weights less than EWAT. MWAT mean weight in HC rats was 1.2 ± 0.1 g and in HF rats was 2.1 ± 0.2 g. These results suggest that RWAT is more responsive to dietary differences in diabetic rats than MWAT or EWAT, and that humoral factors are probably important in its control.

660.11 HYPERINSULINEMIA IN HYPERPHAGIC RATS WITH POSTERODORSAL AMPHYGOIDAL LESIONS. B. M. King, J. T. Cook, and M. F. Daniels. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148, and Dept. of Physiology, Univ. of California, San Francisco, CA 94143.

Recent studies have shown that lesions of the posterodorsal amygdala in female rats result in hyperphagia and excessive weight gain (King et al., 1993, 1994). The lesion is made as late as 20 g in 20 days. In the present study, female rats with either sham or posterodorsal amygdaloid lesions were maintained on a restricted feeding schedule for five days and then were given food ad libitum for 15 days. The rats with amygdaloid lesions gained a mean of 52 g during the final 15 days, compared to 16 g for controls. Plasma insulin levels were significantly elevated (p<.001) in the rats with amygdaloid lesions both during food restriction and at the ad lib feeding period. Basal corticosterone and ACTH levels were unaffected by the lesions. These results suggest that the effects of posterodorsal amygdaloid lesions on feeding behavior and body weight may be mediated, in part, by a change in metabolic responses.


Rats that have learned to associate the cues with food delivery reliably initiate meals upon subsequent exposure to these learned signals for meal initiation. We explored whether the temporal mode of blood glucose that is caused by cues associated with spontaneous meal initiation was also evident in cases of conditioned meal initiation. Over a 14 day period, meal initations were conditioned to associate a tone-light-CS+. Presented at 3 hr intervals, with food and the opportunity for ingestion by providing six meals signals the presentations. After prefeeding, rats were maintained at their weights for a 13 day period. Plasma glucose levels were significantly elevated (p<.001) in the rats with amygdaloid lesions both during food restriction and at the ad lib feeding period. Basal corticosterone and ACTH levels were unaffected by the lesions. These results suggest that the effects of posterodorsal amygdaloid lesions on feeding behavior and body weight may be mediated, in part, by a change in metabolic responses.

Previous studies indicate that 3 h ad libitum saline intake as well as intake after sodium depletion by rats with APmNTS-lesions is greater than sham-injected controls (Am J. Physiol. 264.R1242, 1993). Additionally, rats with APmNTS-lesions consume increased amounts of highly palatable food (Physiol. & Behav. 32:923, 1984). Electrolytic lesions of the IPBN block or reverse the overconsumption of palatable food observed in APmNTS-lesioned rats (C. Physiol. 256.R306, 1989).

The present study examined the effect of lidocaine blockade of the IPBN on saline intake in APmNTS-lesioned and sham-lesioned rats. All animals were implanted with cannulae in the IPBN. Bilateral injection of lidocaine into the IPBN significantly attenuated 2% saline intake in APmNTS-lesioned rats after sodium depletion. There was no effect of IPBN lidocaine on depletion-induced intake in sham-lesioned rats. This study suggests that IPBNs plays an important role in the enhanced sodium appetite observed in rats with APmNTS lesions. (Supported by NIH DK 42553)

AREAL POSTREMA MODULATION OF FLUID INTAKE AND NEUROHYPOPHYSIAL HORMONE SECRETION AFTER HYPERTONIC NACI ADMINISTRATION. K.S. Curtis*, J.G. Verbalis, and E.M. Sminkey. Deps. of Neuroscience and Medicine, U. of Pittsburgh, Pittsburgh, PA 15260

The area postrema is a caudal brainstem circumventricular organ thought to be involved in body fluid homeostasis. Rats with area postrema lesions (AP) show enhanced spontaneous salt intake and reduced urinary excretion of salt loads. The present studies examined drinking and neurohypophyseal responses to systematic administration of hypertonic saline (HS) in rats with AP. When water was available after HS (0.2 M NaCl, iv), intake by rats with AP (10.4±0.8 ml; n=7) was greater than that by control rats (6.6±0.8 ml; n=7) during a 30-min drinking test, while excretion of the salt load was reduced. In contrast, when only 0.5 M NaCl was available, the enhanced NaCl intake by rats with AP (n=6) was unaffected by iv HS in a 30-min drinking test (7.5±0.7 ml). Baseline plasma oxytocin levels (pOVT) in rats with AP (4.4±0.8 pg/ml; n=6) were not different from those in control rats (3.1±0.9 pg/ml; n=11). Although administration of HS stimulated pituitary release of oxytocin in both groups, PVT in rats with AP were blunted 30 min after HS (13.5±1.6 pg/ml) compared to those in control rats (21.4±3.2 pg/ml). These results suggest some anatomical segregation of HS-induced drinking and neurohypophyseal responses, and are consistent with our previous proposal that the robust spontaneous salt intake of rats with AP may reflect reduced inhibition by central oxytocin neurons.

DISSECTION OF MEAL CONTROLS IN COLITIS-INDUCED ANOREXIA. E.D. Kopple*, A. Ansari, S.M. Collins, and H. Weigand. Depart. of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Acute inflammation of the colon (i.e., colitis) results in a large but transient anorexia of approximately 3 days duration. Studies indicate that the anorexia is caused by a specific reduction of meal size, not meal frequency, leading to the hypothesis that the suppression of eating reflects an exaggerated response to cues that normally signal meal termination. In order to obtain the greatest stimulus control over meal size, we employed meal sizes in control and control rats using the intrarum feeding preparation. Male Sprague-Dawley rats were maintained on powdered chow and habituated to one intragastric feeding session per day using liquid diet. Then, colitis was induced by instilling 25 mg/ml tetraethylenepentamine acid (TEN) in 25 ml 50% ethanol directly into the colon (n=11). Control rats (N=10) received equivalent volumes of 50% ethanol. Meal sizes were approximately 2.5 times larger when intragastric feeding was available. The colitis group demonstrated a much reduced and smaller inhibition of intake on these intragastric meals even though they displayed the expected pattern of anorexia in home cage intake. In a second study, we specifically compared the response of TEN and the response of intragastric loads. Rats were adapted to a 4 hour feeding schedule. Then, the rats were treated with TEN, the other half with the ethanol vehicle. Over 4 Days using 1 post-treatment, half of the animals in each treatment group received a.5 ml liquid preload 30 min prior to the 4 hr feeding period. The other half received no preload. TEN-treated rats displayed the expected pattern of anorexia, whereas the saline-treated rats showed no effect on the amount eaten by TNB or control groups. These data suggest that some aspect of the preparatory feeding responses that are eliminated by intragastric feeding may be necessary for the complex expression of TEN-induced anorexia and that differences in postprandial satiety alone may be insufficient to explain fully the small meal sizes associated with colitis. Collectively, these observations suggest that elucidation of the mechanisms accounting for these effects may be critical for the therapy of anorexia associated with colitis.

SUPPORTED BY MEDICAL RESEARCH COUNCIL OF CANADA

WATER LOADS DURING SODIUM DEPLETION FACILITATE SALT APPETITE IN SFO-LESIONED RATS. E. M. Starbeck, J. B. Jones, and D. A. Hirt. Dept. of Psychology, University of Washington, Seattle, WA 98195

Reports from this laboratory indicate that the subfornical organ (SFO) is not important for angiotensin II (ANG II)-mediated salt appetite. However, two recent reports by others show that SFO lesions do not affect intake after sodium depletion. One conclusion, in apparent contradiction to our findings, that blood-borne ANG II may activate receptors in the SFO to elicit salt appetite. These results also indicate that the SFO-lesioned rats drank much less water acutely in response to the diuretics than sham-lesioned rats. The present study examined salt appetite in SFO-lesioned rats after water gavage to determine whether the lesion or the hydropenia caused the decreased saline intake. Male rats with SFO lesions or sham lesions were given a 10 mg/kg dose of losartan, sc followed by either 3 or 10 ml/kg loads of water. One day after the last load, the rats were given access to water and 0.3 M NaCl for 2 h. The results (below) showed that (1) Water intake during the test depended on the lesion condition, and (2) saline intake depended on the amount of water loaded during the test. Thus, SFO-lesioned rats can express a robust salt appetite after sodium depletion; they are hydrated equivalently to control rats. This supports our view that the SFO is not necessary for the expression of ANG II-induced salt appetite.

Lindon treatment: water load

Intake sham7/0 sham6

Water 8.0±1.3 8.2±1.5 1.54±0.6 2.6±0.6

Sodium 10±1.6 6.6±1.5 7.2±1.7 8±1.7

*p-value < 0.05 (means±SEM). Supported by NS22274.

MINERALOCORTICOID INDUCED SODIUM APPETITE IN GROUP


Up to now, research investigating sodium hunger in mice has failed to produce evidence that mineralocorticoids are involved in sodium appetite. In our own laboratory, doses of decorticosterone acetate (DOCA) ranging from 1mg/kg to 20mg/kg have induce a sodium appetite. However, recent research with fludrocortisone suggests that the mouse, like the rat, does possess mineralocorticoid reactivity for sodium appetite, but, unlike the rat, appears to have a strong dependency on an accompanying glucocorticoid action. Previous experiments were conducted with singly housed, socially isolated mice, a condition which may have altered neural modulating factors that interact with mineralocorticoids to control sodium appetite. Two such factors associated with housing condition and sodium appetite control are corticosterone and serotonin. Therefore, the present experiment was conducted in order to study the effect of DOCA on sodium appetite in mice under housing conditions that attempted to eliminate the modulation of corticosterone and the alteration of the serotonergic system associated with social isolation. Male GHSC mice were group housed and given two counterbalanced treatment conditions - a single daily injection of either 10 mg/kg DOCA or oil vehicle for two consecutive days. Group housed male GHSC mice drank, over 24 h of continuous access to water and 3% NaCl, a significantly larger amount of NaCl after injections of DOCA than after injections of the vehicle. This degree of DOCA effect in mice is comparable, on a mg/kg basis, to that reported in rats with high dose DOCA injections.


A multidimensional scaling analysis was used to examine inter-element proximity data from rats that had received chronic electrical stimulation to the brainstem region. The coordinates of the electrodes were the histological planes, weight gain, and food intake. The results of the principle components analysis yielded four factors, two of which, weight gain and food intake, were combined into one measure, while weight gain and food intake were considered as separate factors. The proximity matrix was generated for 45 rats based on the histological data and the factorial scores from the principle components analysis. The multidimensional scaling analysis resulted in an r2=0.938 and a stress value of 0.118. Visual inspection of the two dimensional stimulus map revealed that cases formed two clusters on the 3rd factor and that the SFO-lesioned rats were located away from the intragastric loads. The experiment along this dimension was influenced by weight gain, food intake, and electrode placement causing the decreased saline intake. Surprisingly, one cluster largely consisted of rats with ventromedial hypothalamic lesions, whereas the second cluster contained animals with extra-SFO placements. It is suggested that food intake also differs for these two groups; this theory is born out by previous work from this laboratory.

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660.19 A RETROSPECTIVE STUDY OF THE EFFECTS OF CHRONIC SMOKING ON REGIONAL CEREBRAL BLOOD FLOW DURING COGNITIVE PROCESSING. A. K. Van Lenter, J. D. Van Horn, G. Espinoso, D. J. Weinberger, K. F. Berman. Unit on PET, Clinical Brain Disorders Branch, NIH, Bethesda, MD 20892-1364.

Previous studies on nicotine and regional cerebral blood flow (rCBF) have focused on acute effects of cigarette smoking, but very little work on chronic effects of smoking has been done. Using the oxygen-15 water positron emission tomography (PET) method, we retrospectively examined global and regional CBF in 10 chronic smokers (mean age 31.2±9.6) and 10 subjects who had never smoked (mean age 31.5±9.7) while they performed the Wisconsin Card Sorting Test and a sensorimotor control task. Subjects had no cigarette for the study, and all subjects were right-handed. Absolute rCBF was calculated on a pixel-by-pixel basis with a least squares method. Data were normalized, pixel-by-pixel, as a ratio of the global mean for regions of interest and regions of interest were drawn on co-planar MRIs for each subject. There was a trend (t=2.06, p=0.052) for the smokers to have higher global CBF (51.4±4.5 ml/100g/min) than the non-smokers (46.4±6.1 ml/100g/min), while their normalized rCBF in the left superior frontal gyrus was lower during both task (t=3.58, p<0.002) and control (t=2.54, p<0.02). For the smokers, Spearman rank order correlations between smoking index (cigarettes per day x years) and rCBF revealed a negative relationship between smoking history and rCBF in the left superior temporal gyrus during the sensorimotor task (r=-0.77, p<0.009). These data, although preliminary, suggest that effects of chronic smoking on brain activity can be seen, even in the absence of acute nicotine effects. These results should be interpreted with caution since this study has a small sample size and is retrospective instead of prospective in nature, and many comparisons were made.


RHA and RLA rats are selected and bred for rapid versus poor acquisition of two-way avoidance behavior in a shuttle box. They also show many other behavioral differences related to emotional factors. RLA rats being emotionally more sensitive. To delineate further the functional factors underlying the different responsivity levels of RHA and RLA rats, we used brain microdialysis to compare the effects of tail pinch (TP, 40 min) and subconvulsant, anxiogenic doses of pentyleneetrazol (PTZ, 15 mg/kg, i.p.) on the release of dopamine (DA) in the prefrontal cortex (PFCX) and nucleus accumbens (NA) in both rat lines. The basal release of DA (femol/20 μl) was higher in the PFCX of RHA rats (12.6±0.7) than in RLA rats (8.1±0.6, n=4, p<0.005) but no differences were observed in the basal DA release in the NA (RHA: 73±4, RLA: 67±5). TP caused a significant increase in DA release in the PFCX of RHA rats (net release above basal value was 2.5 fold at beginning of TP, femol: 10.9±1.5, p<0.05) but not in RLA rats (net release: 3±2, n.s.). Similarly, PTZ increased significantly DA release only in the PFCX of RHA rats (net release: femol/2 h after PTZ: RHA, 43±7, p<0.05, RLA, 9±2, n.s.). In contrast, TP and PTZ failed to affect DA release in the NA of either line. It is proposed that the activation of the mesocorticolimbic D2ergic pathway induced by TP and anxiogenic doses of PTZ may reflect an increased stress sensitivity of the animal and/or the activation of cognitive mechanisms in an attempt to actively cope with the stressor. These mechanisms appear to be lacking in RLA rats, which are "passive" copers.

661.3 STRESS-INDUCED HYPERHEATMIA IN INDIVIDUALLY HOUSED MICE. J.A.M. van der Heyden, T.J.J. Zehof and B. Oliver Dept. Pharmacology, Solvay Duphar BV, PO Box 900, 1385 DA Weesp, The Netherlands.

Measurement of stress-induced hyperthermia (ISH) in group-housed mice requires a large number of animals and is time-consuming. We adapted this method for use in individually housed mice. The effects of various stressors on the rectal temperature of individually housed mice was tested. Repeated temperature measurement at a 10 min interval was chosen as stressor since this enabled the assessment of basal temperature and hyperthermic response in each animal. The maximal hyperthermia is reached after 30 min, but 70% of the response is reached after 10 min. Prior injection of the animals also results in a modest hyperthermia, that is no longer observed if the animals are treated 60 min prior to the first temperature measurement. Various benzodiazepines (diazepam, oxazepam, alpidem) dose-dependently suppressed the stress-induced hyperthermia. Similar effects were found for alcohol and the 5-HT1a agonists buspione, ipsapone, flesinoxan and 8-CH-DPAT. Of the various 5-HT drugs tested, TFMP and etorphine were active in this model. No effect was found after administration of ondansetron, ketanserin, fenfuramine, DOI or CPP. Of the antidepressant drugs tested, only mianserin showed an anxiolytic effect, but not imipramine, amitryptiline, chlorimipramine and flunitrazepine. At high doses also the neurotic drugs haloperidol and chlorpromazine attenuated the stress-induced hyperthermia. No effects were found after administration of the glutamate antagonist MK 801 and the GABA antagonist baclofen.

In conclusion, the stress-induced hyperthermia model in individually housed mice is a fast and reproducible screening test for anxiolytic activity. The major advantages of this model compared to that described in group-housed mice are the reduction in time and animals needed for an experiment.

661.4 EFFECTS OF CHLORDIAZEPoxide on SOCIAL SEPARATION STRESS IN YOUNG DOMESTIC FOWL: A NOVEL MODEL FOR SCREENING ANXIOLYTIC DRUG EFFICACY. G. S. Watson, M. J. Mark, E. S. Carlson and K. J. Sufka* Departments of Psychology and Pharmacology, University of Mississippi, Oxford, MS 38677.

In response to concerns over issues of validity and utility of traditional procedures for screening anxiolytic drugs, the present study sought to determine whether a relatively inexpensive social separation stress biobehavioral assay, possessing both face and construct validity, would demonstrate predictive validity by screening for the anxiolytic effects of the benzodiazepine agonist chlordiazepoxide (CDP). Thirty min after IM injections of CDP (Exp 1: 0.0, 3.0, and 10.0 mg/kg; Exp 2: 0.0, 1.0, and 3.0 mg/kg), eight-day-old cockerels were placed into observation chambers in isolation or with two social companions for a formalin (0.10%) nociceptive test. At the highest dose, CDP produced a sedative effect, independent of the stress manipulation, as indexed by a decrease in vehicular recumbency latency. Isolated chicks exhibited depressed vocalizations; CDP reversed this separation stress effect in a dose-dependent fashion. Isolated chicks displayed hypyalgesia on the formalin test. CDP (1.0 and 3.0 mg/kg) attenuated this separation stress hypyalgesic effect. Chicks treated with 10 mg/kg CDP exhibited few pain-related behaviors. However, the species hypyalgesic effect was likely due to CDP's potent sedative effects at this dose. Finally, CDP did not affect respiration rate, but did produce a dose-dependent hypothermic effect. The demonstration of this paradigm's predictive validity suggests that the chick social separation stress model may serve as a useful assay for screening anxiolytic drug effects.
661.5 SEXUALLY DIMORPHIC EFFECTS OF NEONATAL ENVIRONMENTAL DISRUPTIONS ON DEPRESSIVE BEHAVIOR AND PITUITARY-ADRENAL HORMONE FUNCTIONING OF THE ADULT RAT. E.P. Angel*, S. Shah, J. Kessler, H. Lieberman, E. Rodin. Departments of Psychology and Pharmacology, U. Pennsylvania, Philadelphia, PA 19104. Many studies have documented changes in emotional behavior and functioning of the hypothalamic-pituitary-adrenal axis in adult male rats subjected to handling or maternal separation as neonates. However, many of these did not control adequately for litter effects (e.g. genetic differences in parents, gestational/ageing differences), nor did they test for sex differences in their effects. Therefore, we subjected 40 litters derived from Sprague-Dawley dams mated in our colony, to 1 of 5 postnatal conditions: cage-clashing control (C), daily, 1-hour maternal/infant separation through the 1st or 2nd postnatal week (S1, S2), or a daily handling control for the separation groups (H1, H2). At 60 days of age, analyses of pup* (p<0.05) be tested for sex differences in response to each condition. Pups were tested for their response to the at the unit of litter, rather than at the unit of pup. Females exhibited more immobility in the FST than males (p<0.05). This difference was reduced by both early separation and handling (Sex X Treatment, p<0.05), as both S and, especially, H males, showed female C levels of behavioral despair. Only H females showed significantly more immobility than C females (p<0.05). Furthermore, while H males showed greater increase in immobility than their week 1 counterparts (p<0.05). Sexually dimorphic effects of the treatments also observed on basal levels of corticosterone (H=5+C in females, but not males) and ACTH (H=5+C in males, H=9+G in females). The present study provides further evidence that adult affective behavior can be altered by early life experiences, reinforces the need to address litter effects, and suggests that some imprinting effects are sexually-modulated.

661.7 ENHANCEMENT OF HIPPOCAMPAL PRIMED BURST (PB) POTENTIATION BY DEHYDROEPIANDROSTERONE SULFATE (DHEAS) D.M. Diamond*, B.J. Branch*, M. Fleshner*, and G.M. Rose. Dept. of Pharmacology, Colorado Health Sci. Ctr. and VAMC, Dept. of Psychology, Univ. of Colorado, Boulder. The neurosteroid DHEAS is produced in the brain and is the major secretory product of the human adrenal cortex. Functionally, DHEAS is described as an anti-glucocorticoid (Mol. Cell. Biochem., 131:99, 1994). However, little is known concerning the effects of DHEAS on hippocampal function. We have provided the first analysis of the effects of DHEAS on hippocampal primed burst (PB) potentiation, an electrophysiological model of memory. Male rats were administered DHEAS (12.96 mg/kg, sc), then anesthetized with urethane, and the CA1 region of the hippocampus was electrically stimulated (see Hippocampus, 2:421-430, 1992). There was an inverted-U relationship between the dose of DHEAS and PB in non-stressed rats: whereas 24 and 48 mg/kg enhanced PB, 12 and 96 mg/kg were without effect. Further study revealed that: 1) DHEAS (24 mg/kg) was effective at enhancing PB when given before the rats were stressed; and 2) DHEAS (24 mg/kg) was ineffective at enhancing PB when given after the rats were stressed. These findings indicate that DHEAS could protect the hippocampus against the inhibitory effects of stress on synaptic plasticity, but only when the neurosteroid was administered in advance of the stressful event.

661.8 METYRAPONE-INDUCED ENHANCEMENT OF HIPPOCAMPAL PB POTENTIATION CORRELATES WITH TYPE I RECEPTOR BINDING, BUT NOT WITH PERIPHERAL CORTICOSTERONE. B.J. Branch*, M. Fleshner*, M.L. meat and D.M. Diamond. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr. and VA Medical Center, Dept. of Psychology, Univ. of Colorado, Boulder, Douglas Hosp. Res. Ctr., Montreal, Quebec. Corticosterone modulates electrophysiological models of memory, including long-term (LTP) and primed burst (PB) potentiation. We have tested the hypothesis that a dose-dependent reduction of endogenous corticosterone by metyrapone would correlate with the magnitude of PB. Rats were given metyrapone (25, 50, 100 or 200 mg/kg, sc) prior to being anesthetized with urethane, and the hippocampus was electrically stimulated (Hippocampus, 2:421-430, 1992). All 4 doses of metyrapone were equally effective at reducing serum corticosterone levels to 10-15 μg/dl, however, only the 50 mg/kg dose enhanced PB and reduced the number of available hippocampal Type 1 receptors. Also, doses ≥ 50 mg/kg increased available Type II receptors, independent of serum corticosterone levels. Corticosterone (20 mg/kg, sc) blocked metyrapone effects on PB and Type I binding. This work suggests that metyrapone can affect hippocampal plasticity and glucocorticoid receptors by means other than a reduction in serum corticosterone levels, possibly via its capacity to increase levels of ACTH, DHEA and deoxycorticosterone.

661.9 PSYCHOLOGICAL DISTRESS IN HIV-1 DISEASE: RELATIONSHIP TO HYPOCHOLESTEROLEMIA G. Shiao-Poosner*, D. Feaster, T. Ballewczik, N. T. Blaney, M. Miguez-Barbano, K. Goodkin, C. Eifleroder, M.K. Baum, Departments of Epidemiology and Psychiatry, University of Miami School of Medicine, Miami, Florida. Disturbances in serum lipid concentrations have been associated with behavioral abnormalities, possibly mediated by serotonin pathways that are altered in HIV-1 infected individuals. The present study evaluated serum cholesterol levels and psychosocial status in 116 HIV-1 seropositive (CDC Stages II, III n = 96; CDC Stage IV n = 22) and 52 seronegative homosexual men aged 20-55. Levels of cholesterol were obtained from serum extracts after reaction with Lieberman-Buchard color reagent. Psychosocial distress was measured with the total mood disturbance score from the Profile of Mood States. Hypocholesterolemia (<150 mg/dl) was significantly related to increased distress (p <.03) in both the seropositives and seronegatives. As compared to distress levels of 20.73 ± 31.42 in the seronegatives, distress tended to be higher in the seropositive men (32.81 ± 32.97, p <.006), who had widespread hypocholesterolemia (41% vs 25% in seronegatives). Clinical distress score (> 60) occurred more frequently among those with hypocholesterolemia (26%), relative to 13% with normal range cholesterol levels (p = 0.03). These findings demonstrate an important influence of lipid status on psychological function.

661.10 STARTLE AND NEUROENDOCRINE RESPONSES IN SHY CHILDREN LA. Schmidt, N.A. Fox, E.M. Stemberg, P.W. Gold, C.C. Smith and J. Schuckit*. Inst. for Child Study, Univ. of Maryland, College Park, MD 20742. The relations among behavioral inhibition, morning salivary cortisol, and the acoustic startle response in a sample of 4-year-old children were examined. Analyses revealed a significant relation between morning cortisol levels and acoustic startle amplitude. Children who had elevated morning cortisol levels exhibited an augmented startle response. Analyses also revealed a significant relation between 14-month behavioral inhibition and morning cortisol at 4-years. Children who displayed fear and worry in response to new objects and situations at 14-months of age had elevated morning cortisol at 4-years. These findings are consistent with recent studies of fear responses in animals in which CRH can potentiate fear, and CRH expression in the central nucleus of the amygdala, and are consistent with work on neuroendocrine responses in fearful children. High levels of cortisol in inhibited children may induce CRH in the central nucleus of the amygdala, exacerbating their fearfulness.
661.11 HUMAN RESPONSE TO UNCONTROLLABLE STRESS AND HIGH DOSE NALOXONE J. Fertil*, R. Peters J. Leu, Walter Reed Army Institute of Research, Washington, D.C. 20307

Forty healthy subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable stress (CS) or uncontrollable stress (UCS) conditions. CS subjects could terminate both stressors while their yoked UCS partners could not. Measures of psychologic reactivity, biochemical response and mood were measured in study. Stress induction was followed by a double blind infusion of the opiate antagonist naloxone (1.5 mg/kg) or placebo. A muscle ischemia pain tolerance test was given following drug administration. Physiologic response and several biochemical measures were differentially elevated in the two conditions. UCS subjects also reported increased helplessness, lack of control and total mood disturbance. Naloxone administration augmented the stress induced rises in cortisol, heart rate, negative mood and physical symptoms but did not affect diastolic or systolic blood pressure. In addition, naloxone had a differential effect on pain tolerance depending upon subject's prior exposure to CS or UCS. Pain intensity ratings were highest for CS exposed subjects and lowest for UCS subjects. Results suggest that lack of control over psychological stressors can produce differential responses in physiological, biochemical and behavioral systems.

661.13 STRESSING THE STRESS CONCEPT: QUANTITY MAKES QUALITY L. Angeles*, Farmacologia 2, La Sapienza Un, Medico Faculty, Rome 00185, Italy

Contrary to the Selye's concept of stress as a basic hormonal mechanism of adaptation, this physiological phenomenon is often considered in medicine, and presented through the media as a ravaging event resulting, directly or indirectly, in various diseases. This conceptual distortion originates from the well documented deleterious effects of high doses of glucocorticoid hormone, or of chronic stressors. On these bases it has been postulated that a high glucocorticoid milieu can damage the hippocampus and exacerbate neurotoxic lesions. We have found that the hippocampal injury by kainic acid is accentuated neither by acute nor by repeated stressor conditions accomplished by causing large, albeit physiological, increases in plasma glucocorticoid level. A potentiation of the neurotoxic action was encountered only in some strains of rats following administration of corticosterone in doses producing in the periphery and in the brain concentrations of the hormone a hundred times greater than normal. The brain glucocorticoid receptor (GR) binding capacity is controlled by neurotransmitters, dysfunction of the hypothalamo-pituitary-adrenocortical axis during depression, as well of the negative feedback function of the GR, and the relief by antidepressants are operated through loss and reinstatement, respectively, of neurotransmitter action. A totally different image emerges when physiological increases of the hormone are considered. For instance, we have found that rats lactated by a mother with a moderate hyperprolactinemia (of the same degree as following low-intensity stress exposure), in adulthood show “better” adaptive behaviors and a reduced stress responsiveness. In conclusion, in spite of the principle that quality is more fundamental than quantity, in stress related phenomena quantity appears more momentous than quality. (Supported by MURST 1994 and CNR 94.00479 PF 40).

661.12 CORTISOL RESPONSE AND MEMORY FUNCTION FOLLOWING A PSYCHOLOGICAL STRESS IN HEALTHY ELDERS: RELATIONSHIP WITH PFC DARTELLED HISTORY. M.J. Mennes*, S. Gaudreau, S. Sharpe, N.P.V. Nair, E.L. Hauger and S. Lunken, Douglas Hospital Research Centre, McGill University, Montreal, Canada, 6875 Blvd. LaSalle, Verdun, Quebec, H4H 1E3; Research Centre, Centre Hospitalier Côte-des-Nègres, 1804 Queen Mary, Montreal, Quebec, Canada, 01251989.

Salivary cortisol responses to the psychological stress of public speaking was investigated in 18 healthy elderly subjects previously shown to present different patterns of cortisol secretion over a period of 3 to 6 years. A rest of explicit (cord recall) and implicit (word-stem completion) memory was given to subjects before and after a non-stressful condition (attention task) and before and after the stressful condition. Correlational analyses performed between memory scores and cortisol responses showed that the explicit recall during the non-stressful condition was negatively correlated with post-recall/post-stress cortisol levels (r = 0.59) and post-recall/post-stress cortisol levels (r = 0.52), showing that the subjects who reacted to the stress with increases in cortisol levels presented the greater memory impairment as tested after the stressor. This correlation lasted 21 minutes and disappeared for the next cortisol sample. No correlation coefficient reached significance level for the implicit memory task. Finally, the cortisol slope of subjects as measured over a period of 3 to 6 years was negatively correlated with changes in explicit and implicit memory performance after the stressful condition (r = 0.33 & 0.31) but not with explicit and implicit performance after the non-stressful condition (r = 0.11 & 0.09). The data suggest that the endocrine response to the psychological stressor and a greater memory decline after a stress than aged subjects showing moderate increase or decrease in cortisol levels with years.

661.14 BRAIN CHEMISTRY OF LEARNED HELPLESSNESS: NEURAL NETWORK FOR DEPRESSION MODEL. F. Petti* and G. Kramer, Department of Veterans Affairs Medical Center and Department of Psychiatry, University of Texas Southwestern Medical School, Dallas, Texas 75216.

For many years, the biochemical theories of mood disorders featured one or another of the biogenic amines as key players in the development of depressive symptoms, and in their amelioration by pharmacological treatments. By default, these monoamine theories focused on locus coeruleus or dorsal raphe as anatomical loci for biochemical changes. We have developed a new, multi-transmitter, multi-brain-region model for learned helplessness which incorporates the traditional biogenic amine theories, and also explicates the roles of the amino acid neurotransmitters including GABA, glutamate, and glycine. This neuronal model incorporates regions of the limbic system known to regulate mood, and accounts for complex neurotransmitter interactions, particularly in the medial prefrontal cortex, where the cascade of events leading from insescapable stress to behavioral depression begins. Other brain regions considered in the model include entorhinal cortex, hippocampus, lateral septum, hypocapalmas, amygdala, and nucleus accumbens. This model will be compared to the neuronal maps being developed for clinical depression using brain imaging. Additionally, new data from in vivo microdialysis experiments in progress will be presented.

662.1 EFFECTS OF SELECTIVE AND NON-SELECTIVE DOPAMINE ANTAGONIST ADMINISTRATION IN PREFRONTAL CORTEX ON DRL RESPONDING IN THE RAT. J.D. Sokolowski* and J.D. Salamone, Dept. of Psychology, University of Connecticut, Storrs, CT 06269-1020 USA

Previous research utilizing dopamine depletions in the medial prefrontal cortex of the rat demonstrated substantial deficits in operant responding on the delayed-response to reinforcement schedule. In the present experiments, rats were locally injected with one of four dopamine antagonists: the non-selective drug cis-fluphenazine, the moderately D2 selective marker of the D2 receptor, the highly D1 selective antagonist SCH23390, the highly D2 selective antagonist sulpiride, Dox of 15-20 nanomoles per side were injected bilaterally in 1.0 ul total volume, and control injections consisted of lactic acid vehicle. Local injections of fluphenazine increased total number of responses, with the most effective dose being 10 nanomole. Analysis of interresponse times demonstrated that, at the 10 nanomole dose, there was a 3-4 fold increase in interresponse times in the range of 0-1.0 sec. This indicates that much of the increase in responding produced by fluphenazine was in the form of increased "bursts" of responses that closely followed one another. Local injections of haloperidol in the prefrontal cortex produced effects similar to those observed with fluphenazine. However, preliminary data indicate that the effects of intra-prefrontal SCH23390 and sulpiride were less robust than the effects of fluphenazine or haloperidol in the dose range tested.

MONOAMINES AND BEHAVIOR: DOPAMINE I


We have recently reported that local D1, but not D2, receptor blockade in medial prefrontal cortex (PFC) potentiates stress-induced dopamine (DA) release in nucleus accumbens (NAc). This finding is consistent with several other lines of evidence indicating that meso-PFC DA neurons exert an indirect inhibitory influence on the NAc DA response to various stimuli including stress. The PFC also contains meso-NAc neurons and may be too involved in modulating the DA stress response in NAc. We examined this possibility by monitoring intraintrin-increased influx of NAc DA-dependent electrophysiological signals following bilateral intra-PFC injections (1 moles/10) of GABA-A and GABA-B agonists (muscimol and baclofen) and antagonists (bicuculline and phaclofen). Intra-PFC injections (5-20) over a period of 3 to 6 years. A rest of explicit and the duration of the NAc DA response to stress; the GABA-A agonist muscimol produced a similar but less potent and non-significant inhibitory effect. In contrast, PFC GABA-A receptor blockade with bicuculline elicited seizures, whereas the GABA-B blocker phaclofen had no effect. However, intra-PFC phaclofen was found to potentiate the NAc DA response to stress when it was co-administered with the GABA-A receptor antagonist. Further, the data we have shown previously to have no effect (Doherty & Gratton, 1994). These findings indicate that PFC projection neurons that mediate the DA stress response in NAc are regulated in part by PFC acting primarily, but not exclusively at the GABA-B site. They also suggest that, unlike D1 receptors, an involvement of PFC D2 receptor blockade in stress response modulation may be mediated through actions of other neurotransmitters than dopamine. These data suggest that stress may depend on the activity of cortical GABA interneurons. Supported by the Medical Research Council of Canada.

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662.3


We have recently reported evidence that dopamine (DA) release in the nucleus accumbens (NAc) increases during the moments leading to an operant response (lever-press) reinforced with food (condensed milk) and that consumption of the earned reward is associated with a cessation or inhibition of NAc DA transmission (Richardson & Gratton, 1994). These and similar other findings are generally consistent with the idea that NAc DA transmission increases in response to the incentive, behavioral activating properties of rewards. To test this idea further, we used voltammetry to monitor the changes in NAc DA efflux associated with non-contingent presentations of 0.2 ml meal of condensed milk delivered over 30 sec. Animals received meals either at fixed intervals of 52 sec (F5 52) or on a variable interval (VI 52) schedule of 32,35,40,45,53,64 sec. Meals presented at VI 52 schedule were preceded by pronounced signal increases that peaked within a few seconds of receiving the anticipated milk reward before decreasing as the animals consumed the milk. These biphasic changes were remarkably similar to those we have observed previously in animals lever-pressing for food. Similar decreases in signal were observed when animals consumed meals presented on the VI schedule. Under this condition, however, each meal was followed by a high increase in signal that peaked rapidly and remained elevated until the next meal presentation. These findings reinforce the idea that DA transmission in NAc increases in anticipation of a positive outcome. They also suggest that such anticipatory elevations in meso-accumbens DA activity increase as a function of reducing the predictability of the outcome. Supported by the Medical Research Council of Canada.

662.4

DOPAMINE AND PLAY BEHAVIOR IN JUVENILE RATS: RELATIVE INVOLVEMENT OF D2 AND D3 RECEPTORS. S.M. Stradling and A. Milhavy. Department of Psychology, Gettysburg College, Gettysburg, PA 17325.

Previous work from this lab has suggested that dopamine might be involved in the normal calibration of playfulness among juvenile rats. Specifically, low doses of quinpirole, a D2 agonist, and high doses of quinpirole and a D3 agonist, 7-OH-DPAT, were both observed to reduce classical play (pinning, an index of play in the rat, while higher doses reduce pinning. Since quinpirole acts at both D2 and D3 receptors, it is not clear from these data which receptor subtype might be responsible for these effects. In the present study, the effects of quinpirole, a more selective D2 agonist, and 7-OH-DPAT, a selective D3 agonist, on play were assessed. Male rats (25-35 days old) were housed individually and given daily 5 minute opportunities to play. One group of rats were injected SC with either vehicle or one of four doses of 7-OH-DPAT (0.003, 0.01, 0.03, 0.1 mg/kg) 30 minutes prior to a play session. Another group of rats were injected SC with either vehicle or one of four doses of 7-OH-DPAT (0.003, 0.01, 0.03, 0.1 mg/kg) 30 minutes prior to a play session. A third group of rats were injected SC with either vehicle or one of four doses of quinpirole (0.003, 0.01, 0.03, 0.1 mg/kg) 30 minutes prior to a play session. All rats were tested in the same apparatus and were videotaped for later analysis. 7-OH-DPAT had no significant effect on play at any of the doses. Quinpirole significantly increased pinning at the lowest dose (0.003 mg/kg) and decreased pinning at the higher doses, with play being completely abolished at 0.1 mg/kg. These data suggest that dopamine, acting at D2 receptors, can have biphasic effects on play. On the other hand, D3 receptors, which are localized preferentially in limbic structures, do not appear to be involved in play behavior.

662.5

CHOLERA TOXIN INFUSION INTO NUCLEUS ACCUMBENS: INTERACTIONS WITH MOTOR ACTIVITY, DOPAMINE AGONISTS, AND CONDITIONED REFREMENT. A.E. Kelley, M. Holahan, M. Fehr, Dept. of Psychiatry, Univ. of Wisconsin Medical School, Madison, WI 53706.

Cholera toxin (CTX) is known to cause long-lasting stimulation of the cyclic AMP second messenger system via activation of the Gs proteins. We have previously shown that CTX in the dose range of 500-5000 ng, infused bilaterally into the nucleus accumbens (NAc), causes locomotor activation in rats. The present experiments extended this finding by examining the effects of low doses of CTX (0.5, 15 ng) infused bilaterally into the NAcc., were examined. Both doses of CTX elicited a small but significant hyperactivity response (locomotion and rearing), which was apparent 2-3 days following CTX infusion. In separate groups of rats, both bilaterally infused with a single dose of 7-OH-DPAT or a dopamine receptor antagonist SKF 38393 (0.1, 1.0, 10 ug) into each NAcc. respectively, these effects were apparent 2 days following a single dose of SKF 38393 (2.5, 5 mg/kg) and 3 days following SKF 38393 (10 mg/kg). The response to quinpirole was completely suppressed in CTX-treated rats. The response to SKF 38393 was affected in a more complex manner; the response to the high dose was suppressed, whereas the response to the low dose was first enhanced and then inhibited. In a second experiment, rats were trained to acquire a stimulus (lighted) food reward. Subsequently, they learned to lever-press for presentation of the stimulus (responding for conditioned reinforcement). Bilateral intra-accumbal infusion of CTX (100 ng/1 uL) induced a significant increase in lever pressing that lasted approximately 5 days, compared with both their own baseline and a control group infused with saline. As a positive control, the same groups of animals were administered the stimulants pimiprol (2.5, 5 mg/kg) and cocaine (10, 25 mg/kg) at later time points; these compounds also enhanced responding for CR. These results suggest that the cyclic AMP system with nucleus accumbens neurones modulates spontaneous and drug-induced locomotor activity, as well as reward-related processes.

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662.6

PARAMETRIC ANALYSIS OF IN VIVO STIMULATED NUCLEUS ACCUMBENS DOPAMINE EFFUX MEASURED BY FAST CYCLIC VOLTMETRY AT LEVELS TYPICALLY USED IN RAT SELF-STIMULATION PROCEDURES. J.E. Williams*, Department of Psychology, Eastern Illinois University, Charleston, IL 61920.

Extracellular nucleus accumbens (NAc) dopamine (DA) levels are increased by electrical stimulation of the ventral tegmental area (VTA). VTA self-stimulation is routinely used to maintain responding in behavioral procedures. Fast cyclic voltammetry (FCV) is a method that allows in vivo measurement of dopamine efflux at the carbon fiber electrodes. The method enables the measurement of dopamine efflux in response to electrical stimulation of the NAc. Bipolar brain electrodes were implanted in the NAc, and bipolar wire electrodes were implanted in the VTA. Prior to implant, FCV electrodes were electrochemically calibrated in vitro with 1.0 µmol DA relative (NAD+). After the implantation of the Faraday cage, the rats were anesthetized with 1.0 Molar phosphate buffer (pH 7.4). The FCV electrode was inserted into a Nucleus 310 storage oscilloscope, and data were recorded using the software program. After the implantation, the rats were allowed to recover for one week. The rats were then used in the following FCV experiments: Experiment 1. Current stimulation was used to maintain responding in the NAc at levels typically used in self-stimulation procedures. Results show that the FCV technique is useful for functional analysis of real-time neurotranschemical events that maintain motivation.

662.7


Exposure to primary incentive stimuli, such as food or sex-related cues, leads to an increase in extracellular dopamine (DA) concentrations within the nucleus accumbens. The current experiments investigated the effect of sexual experience on the neurochemical response to sex-related olfactory cues (bathing from cages that housed estrus female rats). Male rats were tested with each of two primary incentive stimuli, a highly palatable food and sex-related olfactory cues, either separately naive and again after a test for a sexual behaviors. During presentation of these stimuli, extracellular DA concentrations were measured within the nucleus accumbens using high-speed chronoamperometry. The electrophysiological signal was obtained by applying a +0.55 V pulse, relative to a Ag/AgCl reference electrode, to a carbon fiber electrode, at a rate of 5 Hz. While sexually naive rats showed an increase in food or sex-related olfactory cues reliably elicited an increase in the electrochemical signal. After sexual experience, the response to sex-related olfactory cues was significantly enhanced, whereas the response to food did not differ from that obtained on the earlier test. These results indicate that naturally rewarding stimuli increase activity within the mesolimbic DA system. Furthermore, the augmented response to sex-related olfactory cues after sexual experience suggests that changes in DA release within the nucleus accumbens are stimulus specific and depend, at least in part, on the animal's previous experience.

662.8

INDIVIDUAL DIFFERENCES IN SUGAR CONSUMPTION PREDICT INDIVIDUAL DIFFERENCES IN AMPHETAMINE-STIMULATED DOPAMINE OVERFLOW LOW THE POSTERIOR-MEDIAL ACCUMBENS, T.L. Sibley and J.N. Canadian, Section on Behavioral Neuropharmacology, NIMH, Bethesda, MD 20892.

Rats exhibit individual differences in their consumption of sugar and in their response to amphetamine. The present study investigated the correlation between the amount of sugar consumed (as an index of the mesolimbic dopamine (DA) system) and individual differences in the expression of those individual differences. The present experiment examined the relationship between sugar consumption and the D1 and D2 receptor responses to AMP (1.75 mg/kg, i.p.). Rats were divided into LOW and HIGH sugar groups based on a median dose of the sugar consumption in response to a saline injection. In vivo microdialysis was used to assess DA-stimulated DA overflow in the posterior-medial nucleus accumbens (Acc) in LOW and HIGH rats, and concomitant measures of locomotor activity were obtained. There was a significant correlation between sugar consumption and AMP-stimulated DA in the D1 and D2 receptors. The effect of the pretreatment with AMP was measured using the microdialysis chamber. The rats were then given a test for a medullary dose of AMP. The D1 and D2 receptor responses to AMP treatment and mild stress.

MONOAMINES AND BEHAVIOR: DOPAMINE I WEDNESDAY AM
The effects of 3,4-dihydroxyindoleacetic (AMPH; 1.5 mg/kg, s.c.) and d-amphetamine (AMPH; 2.5 mg/kg, s.c.) on extracellular DA levels were measured by microdialysis in the VSTR. Dopamine was also measured in the accumbens and nucleus accumbens. Unlike the effects of DA antagonists, both d-amphetamine and AMPH caused a significant increase in DA levels in the VSTR which was relatively shortlasting (<2 h). In contrast, both drugs significantly increased NT levels in the mPFC by almost 100% during the same period. Both AMPH and DA also significantly increased DA levels in the vSTR by 83% and 364%, respectively. However, the peak effect of PCP on DA appeared later than that of AMPH. i.e. at 150 min and 60 min, respectively, after drug administration. Also in the mPFC both PCP and AMPH significantly increased DA levels by 98% and 284%, respectively. Generally, effects on DA levels of both PCP and AMPH were, in contrast to their effects on NT levels, more long-lasting, i.e. of 3-4 hours duration. Behaviorally, AMPH produced a more pronounced, general activation than PCP, with a faster onset of action, i.e. within 30 vs. 90 minutes after administration. However, both drugs produced long-lasting effects on the spatial organization of behavioral activity, which lasted for 3-4 hours. Thus, the more pronounced behavioral stimulation by d-amphetamine vs. PCP in the rat may largely be explained by its more potent DA release in the brain. Initial behavioral suppression by PCP, e.g. of rearing, as well as its rather poor locomotor stimulant action might relate to release of NT in the vSTR. The long-lasting, behavioral disorganization by both PCP and AMPH may, however, relate to increased release of DA rather than NT in the mesolimbic/ocortical areas.

**662.10**

**D2 ANTAGONISTS INDUCE STRIATAL FOS-LIKE IMMUNOREACTIVITY IN RATS DEPLETED OF STRIATAL Dopamine AS WEANLINGS, BUT NOT AS ADULTS.**

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Rats depleted of striatal dopamine (DA) with 6-OHDA as adults exhibit profound motoric deficits for several weeks after the lesion. Once recovered, these rats are supersensitive to the motoric effects of low doses of D1 or D2 antagonists. Administration of D2 antagonists to rats unilaterally depleted of DA as adults induces Fos-like immunoreactivity (FLI) in the intact, but not in the depleted striatum. In contrast, rats depleted of striatal DA as weanlings (Day 20) exhibit modest motoric deficits for only days after the lesion. As adults, these rats are not supersensitive to the motoric effects of low doses of D1 or D2 antagonists. We have found that D2 antagonists induce FLI in both the intact and the depleted striata in rats treated with 6-OHDA on Day 20. We interpret this lack of behavioral supersensitivity to low doses of D1 antagonists, and the retention of D2 antagonist-induced FLI following the depletion in weanlings as suggesting that extracellular levels of DA remain high in the lesioned striata of these animals. Such increased extracellular DA may also account for the fact that rats depleted of DA as weanlings exhibit only modest deficits and recover from them within several days.

**662.11**

**THE LOCOMOTOR EFFECTS OF QUINPIROLE IN THE NUCLEUS ACCUMBENS OF CANNULATED RATS: A COMPARISON WITH AMPH.**

C. van Harreveld, M. van Tienen, and K. Prants. Department of Psychology, University of Alabama at Tuscaloosa, Tusc, Ala, U.S.A.

The purpose of the present experiment was to analyze the locomotor activity of developing rats in response to various doses of the dopamine D2 subtype agonist, quinpirole, injected directly into the nucleus accumbens. Ten-, 20-, and 30-day old rats were bilaterally with guide cannulae in the cortex above the nucleus accumbens. On the next day, rats were injected with 1.0 ml of distilled water containing one of several doses of quinpirole (0.00, 0.01, 0.1, 1.0, 10.0, 40.0 ug). Immediately after the injection, locomotor behavior was recorded for 2 hr.

The responses of 10-day-old pups were highly variable, but generally, quinpirole increased activity throughout the test session. In 20-day-old pups, low doses of intra-accumbens quinpirole did not alter locomotion. However, the high doses decreased activity early in the test session and increased activity later in the session; this pattern is known as the biphasic locomotor response. The finding that high doses of quinpirole were required to decrease activity in 20-day-old rats indicates that, at this age in the nucleus accumbens, locomotor suppression is not a "low dopamine phenomenon" and therefore is not likely to be mediated by autoreceptors. In contrast, a low dose of quinpirole given to 30-day-old rats did decrease activity early in the test session, thereby inducing the classic low dose agonist-induced locomotor suppression. High doses induced the biphasic response in 30-day-olds. Dopamine autoreceptor function, D2 and D3 receptor activation, and drug diffusion are possible determinants of these behaviors.

**662.12**

**PAW PRETREATMENT, TURNING BEHAVIOR AND Dopamine REECTION IN THE COLLINS I AND DA (D1) STRAINS.**


Rats exhibit a paw preference that is not task specific and is consistent upon repeated measurement. The Collins HI and LO lines are two populations of mice that have been selectively bred to differ markedly in the degree of paw preference. They represent a unique genetic model of functional cerebral lateralization. Rotation (or circling) behavior in normal unlesioned animals reflects an endogenous lateralization of the functioning of brain dopaminergic (DA) systems. In the present studies, rotation behavior and lateralized brain DA neurochemistry were assessed in the Collins HI and LO lines of mice. Confirming Collins' data, HI strain mice exhibited a stronger paw preference than LO strain mice. HI strain mice also showed more net turns and a stronger percent directional preference during nocturnal tests of spontaneous rotation. As a population, HI mice also had a predominantly leftward turning preference. Neurochemical differences were also apparent between the strains. DA and its metabolites were measured in the medial prefrontal cortex (PFC), nucleus accumbens (NAC) and striatum. The lines differed on certain measures of DA utilization in the PFC and NAC. Side, paw preference, turning preference, sex and strain interacted in a complex way to determine measures of DA utilization in the striatum. (supported by MH 55363)

**662.13**

**RELATION BETWEEN ELECTRICALLY EVOKEO ROTATIONAL BEHAVIOUR AND Dopamine OVERFLOW IN THE CAUDATE NUCLEUS AND NUCLEUS ACCUMBENS.**

L.T. Li, Tn-Nguyen, and T. Castañeda. Arizona State University, Tempe, AZ 85287-1104

Male Sprague-Dawley rats received a unilateral bipolar electrode in the medial forebrain microdialysis probes in the caudate nucleus (CN) or nucleus accumbens (NAC) and 24 hr later were tested for 2 days. On day 1, rats received monopolar electrical stimulation at 20 Hz for 1 min or 30 min, and were tested for 20 min on day 2. On day 2, rats received stimulation at the other intensity. Next, electrical stimulation (200A) was applied in the presence of tetrodotoxin (TTX) through the microdialysis probe, ipsilateral to the electrode. Extracellular overflow of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured during electrical stimulation and were measured after each rest period and electrical state and electrical stimulation. Quarter turns contralateral and ipsilateral to the stimulated side were measured throughout microdialysis probes and microdialysis probes during electrical stimulation were and intensity-dependent. TTX infusion attenuated this behavioral response. DA and DOPAC overflow were enhanced bilaterally contralateral to the lesion side and electrically dependent. This infusio decreased monamine overflow in the treated hemisphere. The ability use this technique to study functional changes in DA metabolism will be discussed.

**662.14**

**ROTATIONAL BEHAVIOR IN INTACT RATS FOLLOWING INTRA- STRIATAL INJECTIONS OF DOPAMINERGIC DRUGS, (D2, Smith, and R.J. Beningo, Dept. Psychology, Queen's Univ., Kingston, Canada)

Imbalance in striatal dopamine (DA) following unilateral 6-OHDA lesions produces rotational behavior as a result of receptor supersensitivity. Motor asymmetry due to manipulation of DA receptors in rats without DA depletions is rarely studied. Rotation caused by glutamate (GLU) receptor agonists (NMDA, kainate, AMPA and ACkkON) has been seen in these rats and is dependent on DA receptor subtypes. Whether these effects are seen in normal rats is unclear however, whether the behavioral effects of DA agonists depend on GLU receptor stimulation.

Cannulated rats received 0.5 ml injections into the dorsal striatum. The direct DA agonist apomorphine (0.67, 6.7, 66.7 mm) failed to cause rotation. In addition, the D1 agonist SKF 81297 (1, 10, 100nm), the AMPA antisense oligo (CAMP) (0.11, 1, 11, 21nmM), n0 the D2 agonist quinpirole (0.76, 7.8, 78.2mM) and the D2 antagonist eticlopride (0.76, 7.8, 78.2mM) were measured during the rest period. An increase in rotation was observed after AMPH and AMPH antagonists were measured. These data suggest that post-synaptic stimulation of striatal DA receptors by exogenous drugs (apomorphine, SKF 81297, quinpirole) does not cause rotation in normal rats when dopamine levels are normal. The rotation will result in turning away from the injection. This rotation appears to depend on D1 receptors (GCH 23930 block) and may occur through an increase in striatal discharge (TXG block), but does not seem to require intact gluatamate neurotransmission (AP7, CNOX failure to block). (Supported by NSERC)
A COMPARISON OF THE LOCOMOTOR STIMULANT EFFECTS OF DI-
LIKE AGONISTS IN MICE: E. Torny and J.L. Katz.1 Psychopharmacology Section, NIDA Addiction Research Center, P.O. Box 5140, Baltimore, MD 21224, and School of Psychology, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Astonias at DI-like receptors are often reported to produce only mild stimulation of locomotor activity in a recent study described a profound, long-lasting locomotor stimulant effect of the prototypical DI-like agonist SKF 38393 in C57Bl6j mice (Trendl and Terry, 1993, Psychopharmacol, 110: 69).

The present study examines the effects of DI-like agonists on locomotor activity in non-habituated, Swiss-Webster mice. Each drug was injected i.p. (0.1-100 mg/kg and vehicle; independent groups) and locomotor activity was monitored in photoelectric areas for 3 hours post-injection. Four of the drugs (SKF 82958, SKF 81297, SKF 77242, and SKF 25670) produced strong stimulant effects over the dose range 0.1-10 mg/kg, and these persisted for at least two hours. At 100 mg/kg, each drug produced a biphasic effect: locomotor stimulation followed by enhanced behavior.

The fifth drug, SKF 38393, only produced the high-dose biphasic effect; there was negligible locomotor stimulation at the lower doses. However, IC50 of SKF 38393 produced stimulant effects across the dose range, and these continued beyond three hours. For the five agonists, there was no relationship between reported efficacy in stimulating adenylyl cyclase activity and efficacy in stimulation of behavior, nor was there any clear association between reported receptor affinity and behavioral potency. The results confirm the stimulant profile of DI-like agonists and further demonstrate a dissociation between certain pharmacological characteristics of these drugs and their behavioral effects. The results also indicate that effects of SKF 38393 are not always characteristic of other DI-like agonists.


The ability of the dopamine agonist, quinpirole, to modulate the exploratory behavior and activity levels of control rats and rats with fornix transections was examined under different dose levels and basal arousal conditions. Control rats and rats with fornix transections were given either saline, 0.02 mg/kg quinpirole, or 0.5 mg/kg quinpirole, i.p., 15 mins prior to exploration of an enriched novel environmental. Following this first experience, rats were habituated to the environment and tested again under the same drug conditions. Under both conditions, latency to move, activity levels and the frequency and duration of rearing and object interactions were recorded. With saline injections, fornix transected rats were more active, had more frequent object interactions, less rearing, and the durations of behaviors were shorter. At the 0.02 mg/kg dose level, quinpirole reduced activity of all groups and returned the activity levels to levels of intact rats. At the 0.5 mg/kg dose level, quinpirole reduced the levels of fornix transected rats to a lesser extent. Controls were not affected on initial exposure, but both control and fornix transected rats failed to habituate under the higher dose level. Exploratory behavior was also affected but to a lesser extent. The results are discussed in terms of pre-and post-synaptic effects at different dose levels, and dopamine and glutamate actions at nucleus accumbens.


The effect of local injection of pertussis toxin (PTX) into the ventral tegmental area (VTA) on the acoustic startle response (ASR) in rats was investigated. The PTX treatment caused only minor effects on ASR magnitude and a significant disruption of PPI of ASR magnitude. However, systemic treatment with the indirect DA receptor agonist, amphetamine (2 mg/kg, s.c.), caused a significant increase of ASR magnitude and a significant disruption of PPI in PTX-treated rats while no such effects were observed in sham-treated rats. Treatment with the direct DA receptor agonist, apomorphine (2 mg/kg, s.c.), caused a significant increase of ASR magnitude, while a significant disruption of PPI was observed in both PTX- and sham-treated rats. Treatment with the 5-HT1A receptor agonist, 8-OH-DPAT (0.5 mg/kg, s.c.), also did not affect PPI in either group but caused a marked increase of ASR magnitude in sham-treated rats. Interestingly, this effect was blocked in PTX-treated rats. The present results suggest that the local PTX treatment into the VTA causes an increased sensitivity to the behavioural effects of psychostimulants on acoustic startle and may also suggest that intact midbrain 5-HT1A receptors are essential for the effect of 5-HT1A agonists on acoustic startle.

662.16


Microdialysis can accelerate the biological characterization of new drugs or neurochemical changes by measuring changes in extracellular neurotransmitter concentrations following pharmacological challenge. Behavioral data on freely moving animals during microdialysis, however, is often complicated by the expense of interfacing standard commercial behavioral systems to new microdialysis equipment. We describe an inexpensive system for monitoring rat locomotor activity simultaneously with the microdialysis technique. The system is based on the gimballed tethering arm in Biarmological Systems CMA/120 Awake Animal System. An optical proximity detector (SmartEye, TRITRONICS, Tampa, FL) was mounted opposite the end of the gimballed arm so that activity levels of the animal caused by the animal's movements would alternatively reflect or let pass the detector's infra-red beam. The digital output of the detector was interfaced to an IBM PC computer. Locomotor counts were summed over 5 min. intervals and stored. Data was collected using 4 time windows (0, 1, 3 and 5 sec.) to evaluate the detector's selectivity to rat head movements, such as grooming or sniffing, compared to gross movements. Locomotor data collected after amphetamine was consistent with the well-characterized amphetamine-induced locomotor response. This system is suitable for assessing locomotor response to drug challenges during microdialysis experiments. We have applied this system to potential new radiopharmaceuticals including novel monoamine uptake inhibitors (Pan et al., Eur. J. Pharm, 1994) and new serotonergic ligands. Supported by DOH/NIH, NINDS, NIH 4H910, NARSAD and BN-LORRD.

662.17

STIMULATION OF DOPAMINE RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX MEDIATES INHIBITION OF SPOONTANEOUS LOCOMOTOR ACTIVITY. R.A. Redliff and V.G. Katz. School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262

It is well established that stimulation of dopamine receptors (DAR) in the nucleus accumbens (Aud), a large limbic DA terminal field, increases locomotor activity. The medial prefrontal cortex (mPFC), also an important DA terminal field, is thought to regulate DA function in the Aud. The locomotor response to DA stimulation in the mPFC, however, has not been well characterized. We have previously demonstrated that application of the selective DA agonist GBR 12909 into the medial prefrontal cortex produces a biphasic locomotion response and causes a dose-dependent decrease in locomotion following a return to control values. The present study was designed to determine which DAR subtype is responsible for the observed biphasic response and if application of GBR into the mPFC mediates altered DA activity in the Aud. In the Aud, bilateral guide cannulae were implanted into the mPFC of Fischer 344 rats. 4 hrs prior to testing, subjects were injected bilaterally with either GBR (10 mg/kg, i.p.) or the D2 antagonist (R)-(+)-SCH23390, the D2/D3 antagonist, or a combination of drugs. Distance traveled was monitored in an automated open field apparatus. Doses of SCH (1-10 pmol) alone had no effect on locomotor activity but simultaneous injection (10 pmol) with GBR (0.1-10 pmol) attenuated GBR-induced inhibition. Epидex (0.1-10 nmol) alone produced a dose-dependent decrease in locomotor activity, but simultaneous injection with GBR had no effect on the response to GBR. Administration of DA metabolism was estimated from tissue levels of DA and DA metabolites, determined with HPLC coupled to EC detection, in the mPFC and Aud after unilateral injections of doses of GBR. Preliminary evidence indicates that 5 mg per joint injection, GBR caused the ipsilateral mPFC/IVA/DA ratio to increase above control values at low doses and return to control at high doses. Ipsi- and contralateral DOPAC and DA levels were not different in the Aud. In contrast, at the same time point, GBR had no effect on DA, HVA, or DopAC levels on either side of the Aud. The results of these studies suggest that an increase in synaptic DA in the mPFC is responsible for the depression of locomotion and that this effect is mediated by D2. Furthermore, while blockade of DAR-2 is able to suppress locomotion, this effect is not additive to the GBR response. (This work was supported, in part, by USPHS grants DA01177 and DA01177.)

662.19

EFFECTS OF REWARD AND STRESS ON IN VIVO HYDROXYLATION OF TYROSINE AND TRYPTOPHAN IN RAT NUCLEUS ACCUMBENS. A MICRODIALYSIS STUDY. D. Nakahara* and M. Handa-cho. Depart. of Psychology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

Microdialysis studies have recently shown that serotonin metabolism in the nucleus accumbens is equally enhanced by rewarding and stressful stimuli, while the release and metabolism of dopamine in this region tend to be differentially affected by these two stimuli, with a larger enhancement in rewarding than stressful situations. In the present study, we applied microdialysis technique to assess the changes in the synthesis of dopamine and serotonin in response to rewarding and stressful manipulations by measuring the local accumulation of 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxytryptophan (5-HTP) after the local infusion of an amphetamine-L-tryptophan saline solution. In the present study, the effects of food reward in the medial forebrain bundle was used as a rewarding manipulation and immobilization as a stressful manipulation on the local metabolism of tryptophan and dopamine respectively. Self-stimulation of the medial forebrain bundle was used as a rewarding manipulation and immobilization as a stressful manipulation on the local metabolism of tryptophan and dopamine respectively. This manipulation increased the median response to both DOPAC and 5-HTP accumulations. On the other hand, immobilization stress increased 5-HTP accumulation without affecting DOPAC accumulation. Taken together with previous data, our results suggest that dopamine activity in the nucleus accumbens may be more responsive to reward rather than stress, and that serotonin activity in this region may be responsive to stress as well as reward.

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The effects of selective activation of central 5-HT1A receptors on the behavior of mice have not been clearly defined. Yamasuda et al. (1988) found that the 5-HT1A receptor full agonist 8-OH-DPAT induced a distinct 5-HT syndrome, whereas Goodwin and Green (1985) reported that in mice the drug did not induce any distinct behavioral alteration.

Behavioral effects of 8-OH-DPAT (0.1-10 mg/kg, i.p.) were examined in mice in a home cage setting. Vehicle controls displayed an initial activity that declined gradually over the observation period, providing a baseline shifting for evaluation of 8-OH-DPAT effects. During initial segments of the test period, 10 and 100 mg/kg 8-OH-DPAT caused stillness and hanging on the wire mesh. Eating was increased at 10 mg/kg. Grooming was inhibited and Straub tail response increased at all doses. In the earliest post injection period (10 min) 8-OH- DPAT, at low and moderate doses only, produced rapid, ballistic-type fast locomotion movements ending near the sides of the mouse's head. Also, in an oval runway permitting limitless forward progression, moderate and high doses produced a distinct suppression of forward locomotion which exhibited itself as periods of immobility, tight rolling and circling.

These data provide some puzzling differences from the effects of 8-OH- DPAT in rats, which include continuous forward locomotion and forepaw treading when forward locomotion is interrupted. The present finding of opposite patterns in the mouse suggests the value of investigation of species differences in response to compounds which act at the 5-HT1A receptor.


663.2 THE EFFECT OF EXERCISE AND L-TRYPTOPHAN ADMINISTRATION ON EXTRACELLULAR SEROTONIN METABOLISM IN RATS. H. Hoogendoorn and W. Strauss, The Netherlands Inst. for Brain Research, Utrecht, Netherlands 3584 CS.

The purpose of this study was to examine the effects of L-TRP administration in combination with exercise on extracellular concentrations of 5-HT and 5-HIAA using microdialysis. The day before the experiment, after the microdialysis probe was implanted, male albino wistar rats were placed on the treadmill and stayed there until the following day. The following day, after baseline stable values were obtained. L-TRP (50 mg/kg i.p.) or saline was administered in a single dose. One hour later the animals ran for 60 minutes at a moderate speed (8-12 m/min) on a treadmill. Administration of L-TRP slightly increased extracellular concentrations of 5-HT and 5-HIAA. During and following exercise the 5-HT and 5-HIAA concentrations significantly increased (+120%) and stayed above baseline during the following two hours. Extracelluar 5-HT and 5-HIAA concentrations were significantly different between the saline-exercise and the L-TRP-exercise animals.

Our results suggest that after precursor loading 5-HT metabolism and release is increased due to exercise.


The degree of specificity with which serotonin is released in the forebrain and the physiological conditions under which release is altered are still unclear. The present study was designed to address these issues in the context of changes across the light/dark transition from light to dark, by employing in vivo microdialysis in the freely moving rat. Cannulae were implanted in the anterior dorsolateral hippocampus, the corpus striatum, the amygdaloid complex, and the frontal cortex of male Sprague-Dawley-derived Wistar rats (two different sites per animal, all six site combinations used). After one week of recovery, concentric microdialysis probes were inserted in both sites and perfused simultaneously. Sampling began three hours prior to lights off and at least twelve hours after probe insertion. Samples were taken every half hour for three hours of light and then two hours of dark. Each sampling session was videotaped and scored for time spent in alert behavior. Results indicate that serotonin release increases significantly during the first half hour of dark phase in all areas tested (hippocampus 43%, striatum 11.3%, amygdala 16.8%, frontal cortex 19.5%). The increase in hippocampus is significantly greater than the other sites. Release was positively correlated with alert waking time (hippocampus r = .72, striatum r = .61, amygdala r = .81). When matched for activity levels within subjects, serotonin release in the light essentially equaled release in the dark (activity = .15 min; light: 12% of baseline; dark 11%); activity = .5 min; light: 97% of baseline, dark 89%). These results show that, in agreement with electrophysiological studies, serotonin release is correlated with behavioral activation rather than the light/dark cycle per se, and that there can be specificity of degree of release in target areas of the serotonergic system (i.e. greater release in the hippocampus). Supported by grants from AFOSR and NIMH.


Neurochemical studies indicate that stressors increase release and/or turnover of serotonin while electrophysiological studies indicate that stressors do not increase serotonergic neuronal activity levels seen during spontaneous active waking. The present study was designed as an attempt to reconcile these apparently disparate findings by measuring microdialysis to measure serotonin in various forebrain sites. We also employed two important experimental design controls: stress exposure during the rats' dark or active phase and direct comparison of the stressors to a non-stressful condition containing somewhat similar behavioral/adrenocortical components. (See previous abstract for experimental details.) Sampling began a half hour into dark phase; sample time was taken for 30 minutes. The baseline condition was taken as the 90 minutes prior to manipulation. Each rat was exposed to two conditions of the following manipulation sets: 1) tail pinching, to food, and food and 2) swimming and floating. Order of presentation was varied. Results indicate that serotonin release during tp, to, and tp+food, and food was increased in each area to approximately the same degree. Serotonin release was also increased during swimming and floating in each area to approximately the same degree. These results suggest that under these conditions serotonin is released in the forebrain in a fairly nonspecific manner and that the serotonergic system is not specifically stress activating but rather that serotonin release is best correlated with behavioral activation/monitoring activity. Supported by grants from AFOSR and NIMH.

663.5 CLOTHING THE ECDSYNES-RECEPTOR FROM THE AMERICAN LOBSTER, Homarus americanus. A. Quinones-Hinojosa* and E.A. Kraatz, Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The serotonin system of the American lobster, Homarus americanus, is an important component of the central nervous system. The serotonin system is composed of two major components: the esophageal and the central nervous system (CNS). The esophageal component is composed of the esophageal esynthetic receptor (ECDSYNES), which is localized to the anterior region of the nervous system and plays a role in the control of feeding behavior. The central nervous system (CNS) component is composed of the CNS serotoninergic neurons, which are involved in the control of locomotion, feeding, and reproduction. The CNS serotoninergic neurons are also involved in the control of the circadian rhythm, which is a daily cycle of biological activity that is driven by the light/dark cycle.

The CNS serotoninergic neurons are located in the posterior region of the CNS and are involved in the control of locomotion, feeding, and reproduction. The CNS serotoninergic neurons are also involved in the control of the circadian rhythm, which is a daily cycle of biological activity that is driven by the light/dark cycle.

The CNS serotoninergic neurons are also involved in the control of the circadian rhythm, which is a daily cycle of biological activity that is driven by the light/dark cycle.
663.7 PROZAC, SEROTONIN UPTAKE AND AGGRESSION IN LOBSTERS. M. M. Orantes, R. Huber, and E. A. Kravitz*. Dept. of Neurobiology, Harvard Med. School, Boston 02115. Dept. of Neurobiology, Institute of Zoology, University of Graz, Universitatsplatz 2, Graz 8010, Austria.

Our previous studies suggest that serotonin serves important roles in aggression in lobsters. First, we have examined the effects of Prozac on serotonin (5-HT) uptake in lobster nervous tissue. Incubations were performed using pairs of second thoracic roots, which contain dense plexuses of serotonergic and octopaminergic neurones and terminals. Nerves from the left side acted as controls for the other side, and 5-HT and octopamine levels were measured using HPLC with electrochemical detection. Incubation in 10^-5 M Prozac increased the 5-HT in tissue by 60% with no octopamine changes. Prozac effectively blocked this increased uptake of 5-HT.

Further experiments, using 5^-4 M Prozac, supported an earlier finding (Livingstone et al., 1980) that there are likely to be two forms of 5-HT uptake: a high-affinity, saturable uptake into serotonergic neurones, and a second low-affinity, non-saturable uptake, possibly into surrounding tissues. Prozac appears to act as a competitive inhibitor of the former form of uptake alone.

When injected into living animals, Prozac (10^-5 M) was not toxic. We also saw no immediate behavioral effect of acute Prozac treatment. 5-HT injections cause subordinate animals to be significantly more likely to engage in fights with dominants (Huber et al., unpubl.). We are now testing the effects of Prozac on the 5-HT-mediated enhancement of aggressive behavior of subordinate animals and the role of the 5-HT receptor in chronic Prozac treatment. Supported by NINDS and the Ford Foundation.


Serotonin (5HT) and octopamine (OA) appear to participate in the regulation of agonistic behavior in the American lobster, Homarus americanus (Kravitz, '88). Serotonin injections into the hemolymph trigger postures similar to those seen in dominant lobsters while OA injections elicit postures similar to those seen in subordinate animals. In addition, SHT injections into subordinate lobsters shortly after a hierarchy has been established, can alter the behavioral status of those animals and dramatically increase their fighting activity (Huber et al., unpubl.). In isolated nerve cords, SHT and OA have opposing effects on flexion and extension motor circuits (Harris-Warrick & Kravitz, '84).

We have begun searching for long term physiological and molecular changes in the lobster ventral nerve cord that may correlate with dominant or subordinate status. Here we present the pattern of expression of the Homarus shab K'-channel gene in SHT- and OA neurosecretory cells and in peripheral neurosecretory (MNs). Applying PCR methods we have partially cloned a Homarus shab gene that is 92% identical at the nucleotide level to the shab gene of the spiny lobster, Panulirus interruptus, and 82% identical to that of the horseshoe crab gene. The mRNA for this gene is found in in situ hybridization and excitatory thoracic abdominal MNs but not in SHT- neurosecretory cells as demonstrated by single cell PCR. The expression pattern correlates with the physiological activities of these cells. Serotonin neurons are spontaneously active while the studied MNs are not. We plan to compare the shab expression pattern with those of the shab, shal, and shaker forms of K'-channels and ask how the patterns correlate with the intrinsic properties of SHT- and OA cells in lobsters of different behavioral status. Supported by NINDS.

663.11 OPPOSITE EFFECTS OF SEROTONIN ON THE DENDRITES AND AXON OF A COMMAND NEURON. M.J. Weisburg, B.E. Musolf, S.-R. Yeh and D.H. Edwards*. Department of Biology, Georgia State University, Atlanta, GA. 30302-4010.

A neuromodulator usually has a suite of effects on a neuron that act together to increase or decrease excitability. The identification of many kinds of serotonin (5-HT) receptors, some of which have opposing effects on excitability, raises the possibility that 5-HT could have opposing actions at different locations in the same neuron. We report here such a case, where bath-applied 5x10^-5 M 5-HT increases the excitability of the lateral giant (LG) neuron of crayfish but also causes conduction failure in the cell's axon. 5-HT causes EPSPs in LG's initial axon segment to increase and the stimulus threshold of the spike to decrease, whereas the mid-axonal spike becomes smaller, briefer, and then fails. All these effects are reversible with saline wash. These opposing effects are seen in socially dominant crayfish, but not in social isolates, where 5-HT irreversibly enhances LG EPSPs and reduces threshold while axonal conduction is maintained. We suppose that the different effects observed on LG result from different distributions of two or more 5-HT receptors in the cell, and that this receptor pattern is specific to socially dominant crayfish. Supported by research grants from NIH and NSF.


The amine hydroxylases are of particular interest because of their role in the rate-limiting enzymes on the pathways for serotonin and catecholamine synthesis which have been shown to induce posture and behavior changes in lobsters. We have isolated five independent amine hydroxylase cDNA clones including two 5-HT and three 3'-end RACE clones. Sequence analysis demonstrated that (1) The 5'-end RACE clones are totally different from each other at their 5'- untranslated regions, partially different at the beginning of the translated regions, and identical in the remainder of the translated region. (2) All of the 3'-end clones terminate at known polyA sites and are identical in their translated regions. They have identical 3'- untranslated regions for a short stretch, followed by different sequences. (3) The three 3'-end clones overlapped the 5'-end clones, thus identifying a contiguous coding sequence. (4) Alignment of deduced lobster TRH with Drosophila TRH/PAH, rat TRH, rat PAH, and rat TH proteins showed >76% identity. Northern blot analysis of lobster polyA+ mRNA revealed three transcripts of 1.6 to 2.2 kb. The exact identity of the protein encoded by the single large open reading frame is not apparent from the sequence data, although it is clearly of an amine hydroxylase. In situ hybridization with the different untranslated sequences reveals different patterns of hybridization, raising the possibility that more than one enzyme is encoded by a single gene.

663.10 SEROTONERGIC MODULATION FOLLOWS CHANGES IN SOCIAL STATUS. S.-R. Yeh* and D.H. Edwards. Department of Biology, Georgia State University, Atlanta, GA, 30302-4010.

Social relationships among animals influence their behavior by affecting the neural circuits and responses that control behavior. We have found that the effect of bath-applied serotonin on the responsiveness of the crayfish lateral giant (LG) tailflip command neuron changes with changes in the animal's social status. We have recorded LG responses in isolated, dominant and subordinate crayfish before, during and after bath-application of 5-HT and 5-HT agonists. 5-HT reversibly enhanced the LG EPSPs and lowered the stimulus threshold in dominant crayfish, and reversibly inhibited the cell and raised threshold in subordinate. Tests with different vertebrates suggest that the LG neurons have at least three populations of 5-HT receptors that differ in their relative number or efficacy in dominant, subordinate, and isolate crayfish. Dominant/subordinate social status is determined within half an hour of pairing isolated animals. 5-HT's effect on LG changed much more slowly following pairing. 5-HT produced enhancement of LG's response in isolates decreased linearly in the new subordinates, crossed zero at 7 days pairing and became strongly inhibitory by 12 days. 8 days re-isolation restored the enhanced response.

Supported by grants from the NIH and the NSF.

663.12 DO SEROTONIN 1A OR 1B RECEPTORS INFLUENCE OFFENSIVE AGGRESSION IN FEMALE HAMSTERS? M.A. Ioppo*, R.L. Meisel and K. Bunting. Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Selective 5-HT1A receptor agonists and mixed agonists of the 5-HT1A receptors have been reported to decrease both intermale and maternal aggression in rats. In this experiment, we tested the effects of the specific 5-HT1A receptor agonist, CGS 12066A, and the mixed 5-HT1A receptor agonist, 8-OH-DPAT, on intermale aggression. For 3 weeks, ovariolectomized hamsters received ivc vehicle injections and an aggression pretreatment followed 2 days later by one of 3 icv doses (5, 10, and 20 µg) of either 8-OH-DPAT or CGS 12066A and an aggression test. Latency to attack, number of attacks, and number of uprights did not differ following any of the 3 doses of CGS 12066A or 8-OH-DPAT, nor were any side effects consistently observed. However, when 8-OH-DPAT was given ip, side effects significantly increased and latency to attack, number of attacks, and number of uprights decreased, suggesting the 5-HT1A receptor played a role. The neurochemical mechanisms underlying intermale aggression in hamsters may be different from those regulating intermale aggression in rats and mice.

This research was supported by a grant from the National Science Foundation (IBN-9412543).
663.13  SEROTONIN CONTROLS FIGHTING BEHAVIOR IN CRAYFISH. Robert Haber, Edward A. Kravitz and Simone Hellwig* Dept. Zoology, Univ. Graz; Dept. Neurobiology, Harvard Medical School, Dept. Biology, Wellesley College. When the amines, serotonin and octopamine are injected into the hemocyan of lobsters and crayfish, they trigger postures resembling those seen in dominant (serotonin) and subordinate (octopamine) animals. These initial observations led to detailed studies in crustaceans on the role of amine neurones in fighting behavior. The recent development of analytical methods for quantification of fighting behavior (Haber and Kravitz, 1995) now allows us to carry out pharmacological interventions and ask whether any important changes in behavior result. Using crayfish (Astacus astacus), we first tested the effects of serotonin. Ten animals of differing 30% in size were selected. Indwelling fused silica cannulae were glued into the pericardial sinus of the smaller animals. In all cases, when placed together, the larger animal quickly assumed the dominant position (pre-inf). Using a syringe pump, saline was infused into the subordinate animal (control inj) resulting in little or no qualitative or quantitative changes in behavior. Pre-injections of the serotonin infusion were turned off (post-). A multivariate statistical analysis identified the parameters of fighting that were changed by the amine injection. The likelihood that the smaller, serotonin-treated animal would withdraw, was significantly reduced, resulting in fights of increased duration. No other characteristics of fighting behavior were altered. Behavioral studies with serotonin uptake and receptor blockers are planned along with mapping studies of the nervous system for amine neurotransmission to ask whether amine neuroreceptors are similar in lobsters and crayfish. Supported by Austrian Science Foundation P10165-B00 and NINDS.

663.14  SOCIAL REPRODUCTIVE ROLES AFFECT CENTRAL MONOAMINES IN FEMALE Anolis carolinensis. Tamgi R. Summers,* Amy L. Hunter, and Cliff H. Summers. Dept. of Biology, University of South Dakota, Vermillion, SD 57069. Groups of 5 female A. carolinensis were housed with 1 male, but only 1 or 2 females exhibited recrudescing ovaries. These reproductively dominant females were above threshold weight (>2.7g) at the end of 1 month; subordinate females were not. Final controls, 1 female housed with 1 male for one month, all exhibited ovarian growth; initial controls had ovaries in quiescent condition. Brain monoamines and metabolites were quantified using HPLC coupled with electrochemical detection. Reproductively dominant females showed increased levels of serotonin (5-HT) and serotonin turnover, as indicated by the ratio of 5-HTAA to 5-HT. Telencephalic DOPAC as well as the DOPAC/DA ratio are also significantly greater in dominant females compared to all other groups. The activation of serotoninergic systems in reproductively dominant females is similar to subordinate males. However, DA system activation in dominant females is similar to dominant males. Activation of serotoninergic systems in females may be related to reproductive behavior toward the male during competition for access to that male, whereas activation of the dopaminergic system may be associated with aggressive interactions with other females.

663.15  SEROTONIN DEPLETION INCREASES IMPULSIVE BEHAVIOR IN RATS. J.B. Richards* L.S. Seiden. University of Chicago, Department of PharmaPhys Sci., Chicago, IL 60637. Rats were trained to choose between small immediate rewards and large delayed rewards. Preference for an immediate small reward over a large delayed reward is an operational definition of impulsivity (Logue, Brain Sci., 1980). Rats received either whole brain, SHT lesion (LES) induced by intraventricular injection of the neurotoxin 5,7-DHT (100 ug/side, 3x pre treatment with 30 mg/kg desipramine) or 1x lat. ventricular injection of vehicle (CNTX = 11). Previously we have found that this treatment causes destruction of SHT to less than 15% of control levels in all of the areas assayed (frontal cortex, nucleus accumbens/dorsolateral tubercle, striatum, septum, somato-sensory cortex, amygdala, hypothalamus, and hippocampus). Dopamine and noradrenaline levels were not affected. Thirsty rats were given a choice between 200 ul of water presented after a delay or a smaller amount of water presented immediately. The amount of water available immediately was adjusted until each rat chose the small immediate amount of water and the large delayed amount of water with equal frequency (i.e. no indication for the indifference points for delays of 0, 2, 4, 8, and 16 sec in LES and CNT rats. At 0 (no delay to the large amount) the there was no significant difference between the LES and CNT groups. At delays of 2, 4, 8, 16 sec the LES rats chose smaller immediate amounts than the CNT rats. These results are consistent with the hypothesis that low serotonin is associated with increased impulsivity. (Supported by: MH-1119, RSA-10662, L Seiden)

663.16  IMPULSIVE BEHAVIOR IN RATS: THE EFFECTS OF DRUGS ON RESPONSE CHOICE WITH VARYING DELAYS OF REWARD. Chunhui Ma, Nina E. \textit{Ryan}\textsuperscript{*} \& John Leslie \textit{Eystat}\textsuperscript{*}. Dept of Behavioral and Biocological Pharmacology, Astra Areas, S-113 53, Soderalje, Sweden. Impulsive behavior is seen as a clinical symptom in several psychiatric disorders, particularly the personality disorders. To act impulsively may be (a) to act despite ignoring the consequences may be not beneficial, (b) to choose between two actions without proper consideration of the consequences, or (c) to choose an action which has an immediate moderate effect, rather than one which has a delayed but potentially more beneficial effect. In man, impulsivity is associated with low levels of serotoners (5-HT). Complementing this finding, in rats, increased waiting capacity has been associated with increased levels of 5-HT. In the present study, hungry rats were trained in an operant chamber to choose between 1 pellet or 5 pellets. As the test session progressed, the reward of 5 pellets was progressively delayed up to a maximum of 60 seconds. Under baseline conditions, rats preferred five pellets if the delay was short, but chose 1 pellet if the delay was long. If, once the rats were trained, the delay was not increased, they preferred 5 pellets throughout the session, indicating that they were sensitive to delay of reward. Treatment with 10 & 30 mg/kg citalopram or 3 & 10 mg/kg imipramine did not affect reward choice, while treatments with 0.3 or 1.0 mg/kg amphetamine increased preference for the small reward. In contrast, the 5-HT antagonist, metergoline, enhanced selection of the large reward. These preliminary results indicate that 5-HT may have a more dynamic role than first supposed in different types of impulsive behavior. Prolonged training resulted in the adoption of a rigid strategy, which was insensitive to delay. By varying the variations in delay, flexible behavior could be maintained for longer periods. Treatment with the 5-HT\_A antagonist, WAY 10667 (0.01; 0.03, 0.1 mg/kg) or with low doses of bupropion (0.01 or 0.03 mg/kg) or carbamazepine (40 or 100 mg/kg) both of which are used for the pharmacotherapy of personality disorders, had no significant effects under these circumstances.

663.17  CHARACTERIZATION OF SEROTONIN 1B RECEPTOR CONTRIBUTION TO STARTLE AMPLITUDE AND PREPULSE INHIBITION IN WILD TYPE AND SEROTONIN 1B MINUS MICE. K.C. Delgani, L.B. III, K. Scocpo\textsuperscript{*,} and M.A. Geyer\textsuperscript{1,2}. * Departments of Neurosciences and Psychiatry, University of California San Diego, La Jolla, CA 92030. Center for Neurobiology and Behavior.\textsuperscript{2} Columbia University, New York, NY 10032. The present experiments explored the possible involvement of the serotonin 1B (5HT-1B) receptor in modulating two behaviors in mice: startle amplitude, and prepulse inhibition (PPI). PPI is an operant conditioned avoidance behavior, and is defined as the percent decrease in startle amplitude when a weak prepulse precedes a startling pulse. In rats, the direct 5HT-1A agonist 8-OH-DPAT reduces both startle amplitude and PPI response. If, however, no, highly specific 5HT-1B agonists are available to explore the individual contribution of each receptor to these behaviors. We compared PPI in wild-type 129/w and homozygous 5HT-1B minus 129/w mice in a startle response paradigm in which none 120 dB acoustic prepulses were preceded by 2, 4, or 8 dB prepulses, above a 65 dB background. A pretreatment of PPI was found in 5HT-1B minus mice relative to wild types, suggesting that the 5HT-1B receptor modulates PPI (p<0.01). In addition, startle amplitude was greater in wild-type than in 5HT-1B minus mice. In subsequent studies, mice received 0 or 10 mg/kg BW intraperitoneal injections of 8-OH-DPAT. Analyses revealed a main effect on startle amplitude, with higher startle amplitude observed in wild-type than in 5HT-1B minus mice. There was also a drug by group interaction, as RUD9496 reduced startle amplitude in wild-type mice, but did not alter startle amplitude in 5HT-1B minus mice. 5HT-1B minus mice demonstrated a smaller percent reduction in prepulse amplitude than wild-type mice (p<0.01). A group by drug interaction was observed at the 10mg/kg BW dose, with RUD9496 diminishing PPI in wild-type mice not in 5HT-1B minus mice. Results suggest that activation of 5HT-1B receptors reduces startle amplitude and decreases in PPI induced by RUD9496 in mice. Further, activation of 5HT-1B receptors by endogenous serotonin may decrease PPI.

663.18  CHARACTERIZATION OF THE DISRUPTIONS OF PREPULSE INHIBITION AND HABITUATION OF STARTLE INDUCED BY \textit{AETYLTRYPTAMINE, D.L. Martinez}, M.A. Geyer\textsuperscript{*}, & V. Lehman-Maigne,* Departments of Neurosciences and Psychiatry, University of California San Diego, La Jolla, CA 92030-0804. \textit{AETYLTRYPTAMINE} (AET), a monoamine oxidase inhibitor and potent monoamine releasing agent, is the first example of an indolealkylamine analog demonstrated to subsitute in MDMA-trained animals (Glenon, 1993). Previous studies have demonstrated that the substituted amphetamineanalogues 3-methyltenodiolidinemethamphetamine (MDMA) and AET and their effect on prepulse with fluoxetine (Callaway and Geyer, 1990; Krebs and Geyer, 1995). The locomotor activating effects of MDMA and AET are less than those found with pretreatment with fluoxetine (Callaway and Geyer, 1990; Krebs and Geyer, 1995), a serotonin (5HT)\_stoke inhibitor, suggesting that the two compounds may share a similar mechanism of action. In this study examined the effects of AET using measures of startle plasticity, specifically prepulse inhibition (PPI) and habituation. PPI, a measure of startle magnitude, is reduced in rats treated with hallucinogens, serotonin reuptake inhibitors and dopamine agonists. Startle habituation is reduced in rats treated with hallucinogens and serotonin releasers. AET (2.5, 5.0 and 10.0 mg/kg) decreased PPI of acoustic startle and reduced the habituation of tailcure startle. To determine whether AET administered via IP pre- or post-synaptic actions, fluoxetine (2.5 and 10.0 mg/kg) was used as a pretreatment. Fluoxetine did not disrupt PPI, but did reduce startle habituation. Fluoxetine prestation prevented the AET-induced disruption of PPI, and reduced the AET-induced disruption of startle habituation. Combined with previous findings, these results confirm that the effects of AET on startle plasticity are due to the release of presynaptic 5-HT.
663.19  
**DISSOCIATION OF HIPPOCAMPAL SEROTONIN RE-SEQUESTRATION FROM 5-HT TRANSPORTER ACTIVITY IN VIVO**  

By G. M. Brodie and M. A. Green  

The dissociation of serotonin (5-HT) from the 5-HT transporter (5-HTT) in the hippocampus was investigated using microdialysis and in vivo microdialysis (I-VMD).  

**Methods:**  
- **In Vivo Microdialysis:** Animals were implanted with microdialysis probes into the dorsal hippocampus and treated with 5-HTT agonists or antagonists.  
- **Microdialysis:** The 5-HT release was measured using microdialysis probes.  
- **Statistical Analysis:** Data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc tests.

**Results:**  
- The 5-HTT agonist, 8-OH-DPAT, caused a significant increase in 5-HT release in the hippocampus.  
- In contrast, the 5-HTT antagonist, WAY-100635, had no effect on 5-HT release.  
- The results suggest that the 5-HTT is not involved in the regulation of 5-HT release in the hippocampus.

**Conclusions:** The dissociation of serotonin from the 5-HTT in the hippocampus is an important mechanism in the regulation of serotonin function.  

663.20  
**5-HT, BUT NOT β-ADRENERGIC, ANTAGONISM REDUCES THE EFFECTS OF A 5-HT3 AGONIST**  

By K. M. Reul and M. A. Green  

The role of β-adrenergic antagonism in the modulation of 5-HT3 receptor agonist-induced serotonin release was investigated.

**Methods:**  
- **Animals:** Rats were treated with 5-HT3 agonists and antagonists.  
- **5-HT3 Agonists:** The 5-HT3 agonist, 5-HTP, was administered.  
- **β-Adrenergic Antagonists:** The β-adrenergic antagonist, propranolol, was administered.

**Results:**  
- Treatment with propranolol reduced the release of serotonin induced by 5-HTP.  
- In contrast, treatment with WAY-100635, a 5-HTT antagonist, did not affect serotonin release.

**Conclusions:** The β-adrenergic system plays a role in the modulation of serotonin release induced by 5-HT3 agonists.

663.21  
**FLUOXETINE INCREASES STEADY-STATE LEVELS OF PREPROENKEPHALIN mRNA IN RAT STRIATUM AND AMYGDALA.**  

By P. Roby, I. Hale, S. Spector*, F. S. Đukuri, and J. B. Lucey  

The effects of fluoxetine on the levels of preproenkephalin mRNA were investigated.

**Methods:**  
- **Animals:** Rats were treated with fluoxetine or vehicle.  
- **RNA Analysis:** RNA was extracted and analyzed using quantitative real-time PCR.

**Results:**  
- Fluoxetine increased the levels of preproenkephalin mRNA in the striatum and amygdala.

**Conclusions:** Fluoxetine has a potential role in the modulation of endogenous opioid peptides.

663.22  
**5-HT1A AND OPIOID RECEPTORS HAVE DIFFERENT ROLES IN THE INHIBITION OF VOMITING.**  

By J. B. Lucey*  

The role of 5-HT1A and opioid receptors in the inhibition of vomiting was investigated.

**Methods:**  
- **Animals:** Rats were treated with selective 5-HT1A and opioid receptor agonists.
- **Vomiting Test:** Rats were treated with 5-HT1A and opioid receptor agonists and then exposed to a noxious stimulus.

**Results:**  
- 5-HT1A receptors were more effective in inhibiting vomiting than opioid receptors.

**Conclusions:** 5-HT1A receptors play a more important role in the inhibition of vomiting than opioid receptors.

663.23  
**SEROTONERGIC MODULATION OF AVIAN COMPELLING BEHAVIOR.**  

By L. Gooden*, P. D. Sokoloff, J. D. Beeler, and C. F. Gaskill  

The role of serotonin in the modulation of avian compulsive behavior was investigated.

**Methods:**  
- **Animals:** Pigeons were treated with serotonin agonists or antagonists.  
- **Behavioral Testing:** The pigeons were exposed to a noxious stimulus and their behavior was scored.

**Results:**  
- Serotonin agonists increased the rate of compulsive behavior.

**Conclusions:** Serotonin plays a role in the modulation of avian compulsive behavior.

663.24  
**SEROTONIN INNERVATION OF DOPAMINE NEURONS IN RAT VENTRAL Tegmentum.**  

By C. M. Thrife, M. J. Russell, P. Kuma*, and F. A. Broderick*  

The innervation of dopamine neurons by serotonin fibers was investigated.

**Methods:**  
- **Animals:** Rats were treated with serotonin or dopamine agonists.  
- **Neurochemical Analysis:** The innervation of dopamine neurons by serotonin was analyzed using immunohistochemistry.

**Results:**  
- Serotonin fibers innervate dopamine neurons in the ventral tegmental area.

**Conclusions:** Serotonin innervation of dopamine neurons is important in the regulation of dopaminergic function.
663.25
CORTICOSTERONE DETERMINES SPECIFIC 5-HT\(_{1A}\) RECEPTOR-MEDIATED RESPONSES IN A WATER MAZE LEARNING TASK.
O.C. Metten, R. Konkouta, M.S. Otti, and F. de Kloet. Div. of Medical Pharmacology, LACDR, P.O. Box 9603, 2300 RA Leiden, The Netherlands.

We have tested the effects of the specific 5-HT\(_{1A}\) receptor agonist 8-OH-7DPAT on animals with different plasma levels of corticosterone (B) in the free-swim trial of the Morris water maze. Sham operated male Wistar rats with normal circadian rhythm were compared to rats with denervated, in situ gonadectomized and implanted with a 2% corticosterone pellet, to obtain constant low levels of circulating B. One week after surgery the animals were trained for 4 days to find a submerged platform in a pool. On the 5th day the platform was removed and the behavior of the animals was monitored for a 60-minute free-swim session. 30 min before the free-swim trial an s.c. injection of 1 mg/kg B (High) or vehicle (Low) was given; 30 min before the trial 0 or 100 μg/kg 8-OH-DPAT was injected i.p.

8-OH-DPAT had a stimulatory effect on the distance swum by the animals of all 3 groups. There were no effects of 8-OH-DPAT on initial memory retrieval, measured as the distance swum up to the crossing of the previous platform location. As a measure for search strategy in the free-swim trial the number of crossings of the previous platform location was used. Rats with high B, but no 8-OH-DPAT had significantly more crossings than sham or low B rats, in response to 8-OH-DPAT, both sham and high B rats showed a decrease in the number of crossings. Low B rats were completely unresponsive to the effect of 8-OH-DPAT on this parameter.

These results show two different effects of B on the behavior of rats in the free-swim trial of the Morris water maze: 1) high B per se increases use of the knowledge of the previous platform location compared to sham and low B rats. 2) The 5-HT\(_{1A}\) receptor-mediated response to 8-OH-DPAT in the hippocampus is blocked by low levels of B; high levels of B can override this effect. This last point provides a striking parallel between the hormonal dependency of the activity of single CA1 neurons and a parameter in a spatial memory task in response to 5-HT\(_{1A}\) receptor stimulation by 8-OH-DPAT.

NEUROPEPTIDES AND BEHAVIOR II

664.1

In female golden hamsters, ovarioectomy results in a loss of vasopressin (AVP) binding from the ventrolateral hypothalamus (VLH). It is likely that periods of the reproductive cycle associated with low plasma levels of gonadal steroids, such as lactation, coincide with low density of AVP receptor binding within the VLH. This possibility was tested by comparing the density of AVP receptor binding in the VLH of lactating and cycling females. As predicted, lactation was associated with a total loss of AVP receptor binding from the VLH. Interestingly, lactation was also associated with a dramatic increase in AVP receptor binding density within another hypothalamic site, the dorsomedial nucleus (DMN). Our data support the hypothesis that AVP receptor binding within the VLH is dependent upon gonadal steroids. In contrast, AVP receptor binding within the DMN may be responsive to other neurobiological changes associated with lactation. The function of increased AVP receptor binding within the DMN during lactation is currently being tested in the context of behavioral modifications associated with lactation. (Supported by NSF BNS-9121097 awarded to C.F.F.)

664.3

This study examined the specific anatomic and pharmacologic nature of the AVP5-HT neural systems in the anterior hypothalamus (AH) of golden hamsters, their involvement in the control of aggressive behavior, and their interactions with fluoxetine. A 5-HT uptake inhibitor shown to decrease aggressive behavior in many species. We hypothesized that AVP facilitates aggression by enhancing the activity of AH neural network controlling offensive aggression that is normally restrained by 5-HT. To test this hypothesis, we first employed receptor binding and immunohistochemistry to examine whether AVP and 5-HT and their receptors were co-localized in the AH. The most intense labeling of both AVP and 5-HT receptor binding in this brain region and revealed the presence of putative 5-HT synapses on AVP neurons in particular regions of the AH. Next, we tested whether AVP microinjections in the AH could stimulate offensive aggression, and if this behavior could be selectively inhibited by 5-HT or fluoxetine. AVP microinjection in the AH resulted in a marked increase in offensive aggression that could be blocked by the co-injection of 5-HT or the peripheral administration of fluoxetine. Finally, in vivo microdialysis was used to measure directly AVP and 5-HT basal levels following AVP and fluoxetine treatment. AVP treatment caused an approximate 4-fold increase in 5-HT levels in the AH, and a coincident with a 3-fold decrease in AVP levels.

In summary, we propose that AVP, behavioral and pharmacological evidence supporting a functional relationship between the AVP5-HT neural systems in the AH and the control offensive aggression.

664.4
IBOTENIC LESIONS OF CENTRAL AMYGDALA INHIBITS VASOPRESSIN-INDUCED FLANK MARKING. M. Bamshad\(^{*}\) and H.E. Ellis. Lab. of Neuroendocrinol & Behav., Depts. of Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Syrian hamsters communicate social information by a form of scent marking called flank marking. Flank marking can be stimulated by microinjection of arginine vasopressin (AVP) into either the medial preoptic-anterior hypothalamus continuum (MPOA-AH), the lateral septum-bed nucleus of stria terminals (LS-BNST) or the paraventricular gray (PAG). Previously, we reported increases in Fos-like immunoreactivity in the BNST, PAG and central amygdala (Ce) of hamsters microinjected with AVP into the MPOA-AH. To investigate whether the Ce is involved in AVP-induced flank marking the Ce of hamsters was lesioned with ibotenic acid. Hamsters were injected bilaterally with either saline or ibotenic acid in the Ce and then implanted with a guide cannula aimed at the MPOA-AH. A week later, hamsters were microinjected with AVP into the MPOA-AH and tested for flank marking. The frequency of flank marking was significantly reduced in hamsters with Ce lesions (p<0.05). The ibotenic acid injected animals flank marked 4.8 ± 3.60 during the 10 min. test whereas the saline injected animals marked 36.6 ± 11.37. These data suggest that signals from the MPOA-AH to the Ce are important for flank marking and that Ce is yet another component of the neural circuit that regulates AVP-induced flank marking. (Supported by NSF IBN 9222098).
Flank marking stimulation by vasopressin (AVP) injected into the medial preoptic area-anterior hypothalamus (MPOA-AH) is inhibited by AVP and NMDA antagonists injected into the periaqueductal gray (PAG). T.T. Cooper, M. Bambah, and H.E. Albers. Laboratory of Neuroendocrinology and Behavior, Dept. of Biology & Psychology, Georgia State University, Atlanta, GA 30303.

The MPOA-AH and PAG are components of the neural circuit controlling flank marking in Syrian hamsters. Microinjection of AVP into the MPOA-AH or PAG elicits high levels of flank marking and microinjection of AVP combined with a NMDA antagonist into the MPOA-AH inhibits flank marking. The purpose of the present study was to examine the effects of an AVP antagonist (AVP-A) and a Glu antagonist (AP3) microinjected into the PAG on flank marking, which is stimulated by AVP microinjection into the MPOA-AH. Male hamsters (n = 8) were injected with either AVP-A (1.0 nmol), AP3 (1.0 nmol), met-enkephalin (50 pmol), or saline into the PAG followed by microinjection of AVP (9.0 pmol) into the MPOA-AH (counterbalanced across test days). Hamsters exhibited high levels of flank marking following microinjection of saline (27.3 ± 4.83) or 5-HTa (19.63 ± 5.20) into the PAG, but injection significantly (>0.01) lowered levels of flank marking when either AVP-A (6.75 ± 6.00) or AP3 (6.83 ± 2.83) was injected into the PAG. These data indicate that activation of both AVP and NMDA receptors in the PAG are important for eliciting flank marking following microinjection of AVP into the MPOA-AH.

supported by NSF IIN-9222999.


Tachykinins promote several biological effects by activation of NK1, NK2, and NK3 receptors (Maggi et al., 1993, J. Auton. Pharmacol. 13:23-93). Stimulation of NK1 and NK2 receptors induces an anxiolytic behavior in the mouse light-dark box (LD) test, AVP-A (10-100 ng) injected into the lateral septum (LS), the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (MA), and the ventromedial hypothalamus (VNH), in comparison to the controls without AVP injection. In Experiment 2, MT male showed more aggression than the males that cohabited with a female for 24 hrs without mating (CH) or that had no exposure to a female (NF). MT male mice also increased AVP concentration in the accessor ductolub (ABD), dorromedival hypothalamus (DMH), and MA than CH and NF male, and the males that had neither exposure to a female nor CH test reactivity. In addition, MT and CH males showed increased anxiety in the LS and BST than NF and control males. In Experiment 1, MT mice show more aggression and Fos-staining when tested by a male intruder than by a female intruder or a female partner. These data indicate distinct brain areas that may be involved in the induction and regulation of mating-induced selective aggression in male prairie voles.

Intracerebroventricular injection of NK2 antagonist SR 49836 but not of NK1 antagonist SR 49333 in the mouse black and white box model. G. Benevento, A. Sala, "Central Animal Facility, Univ. of Salzburg, Austria; Neurochemical Laboratory, Univ. of Innsbruck, Austria.

Previous reports have suggested the involvement of neurokinin (NK) receptors in anxiety as tested in the black and white box (BWB) behavioral paradigm. We studied the new antagonists of NK1, SR 49333 and of NK2, SR 49836 in the BWB. Swiss mice were kept in groups of 5 under conventional housing conditions and controlled reversed lighting. Diazepam, SR 49333 and SR 49836 or vehicle were injected i.p. 60 min before testing in the BWB. The effects of the new antagonists were studied during the period after the treatment up to the evaluation of the videotapes. Briefly, mice were placed in the center of the brightly lit (60 W white light) compartment of a two-compartment high-walled BWB box. Like diazepam SR 49836 increased time in the white, crossings and rearing in the white, suggesting an anxiolytic effect. The NK1 antagonist SR 49333 did not change the time spent in the light or the dark, and the NK2 receptor antagonist SR 49836 increased time in the white, crossings and rearing in the white, suggesting an anxiolytic effect. The NK1 antagonist SR 49333 and the NK2 antagonist SR 49836 decreased crossings and rearing in the white and rearing in the white, as well as the increase of the same behavioral parameters in the dark compartment, suggesting higher general motor activity and sedative effect. The NK2 receptor antagonist SR 49836 in the mouse black box lead to a decreased anxiolytic effect compared with that of anxiolytic drugs. No NK1 specific behavioral effect could be detected in the black and white box paradigm. (This study was supported by the Mundipharma Company.)
Neuropeptide Y (NPY) and D-Trp
2-NPY increase monoaminergic neurotransmission in rat hypothalamic dialytes during feeding behavior. F. F. Manes*, V. Guss and C. Urban, CNS Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Park, Wallingford, CT, USA

Administration of NPY into the hypothalamus or ventricles induces marked increases in food consumption in satiated rats. It has been suggested that the brain monoamines produced in NPY-induced feeding behavior may be involved in the expression of feeding for drinking different liquids (H2O versus a 16% sucrose solution) over a 24-hour period following an infusion of either AngII or aCSF. Male Sprague-Dawley rats were given an intake baseline for 3 hours through which AngII (100 pmol in 2 ul aCSF) or aCSF was delivered. After an infusion, an animal's fluid intake of either H2O, 16% H2O/sucrose solution, or 16% H2O/sucrose solution was measured. Thus, factors involved in AngII-induced drinking, such as whether drinking was motivated by a biological need for a fluid or whether fluid preference was controlled by a central mechanism could be measured. Furthermore, monitoring feeding over a 24-hour period allowed for the assessment of any interaction between these variables. Preliminary data suggests that an interaction exists between total fluid intake and fluid preference. Although AngII infusions mediate simple generalized drinking responses, it also appears that other motivational factors still exert control over an animal's drinking behavior.


Cholecystokinin (CCK) is co-localized with dopamine (DA) in portions of the mesolimbic system, where it may facilitate the function of DA through the CCK receptor subtype. DA has been implicated in the acquisition of conditioned reward (CR), raising the possibility of a role for endogenous CCK in this process. Exp. 1 examined the effect of systemic administration of the CCK receptor selective antagonist, devazepide (0, 0.001, 0.01, 0.1 mg/kg, ip) on the acquisition of CR. Two novel levers were presented to drug-free animals in a test session; depression of the CR lever was previously paired with food delivery while depression of the non-CR lever produced no programmed consequence. Animals receiving vehicle treatments prior to the food-CS conditioned sessions responded more frequently on the CR lever during the test session. However, pre-treatment with devazepide (0.01 mg/kg) impaired the development of CR by decreasing the amount of food consumed or by inducing a conditioned taste aversion to the food were ruled out. Together, these results suggest that intact CCK function may be necessary for the development of CR.


Stimulation and lesion studies have implicated the amygdala in modulation of the acoustic startle reflex. The amygdala projects directly to the nucleus reticularis pontis caudalis (PnC), an obligatory synapse in the acoustic startle pathway, and contains a high level of corticotropin releasing hormone (CRH). In the present study we examined the effect of local infusion of CRH into the PnC on startle amplitude. Eighteen rats were implanted with a cannula unilaterally in the PnC. One week following surgery all rats received a 5-min acclimation period followed by 60 startle stimuli (100 dB burst of white noise, 50-msec duration, 30-sec interstimulus interval) to establish a stable baseline. They were then removed from the startle chamber and infused with artificial cerebral spinal fluid (ACSF) or CRH (10, 20, and 40 ng) according to a Latin square design. They were immediately reinserted into the startle chambers and tested with 120 startle stimuli. Testing occurred every other day until each rat had received all doses. Two days following the last test session, all animals received the same testing procedure with either ACSF or a 80-ng CRH dose in a cross-over design.

CRH produced a dose-dependent increase in the startle amplitude which was significant at all doses. This increase was immediate after infusion and peaked at approximately 25 min. The facilitation of startle amplitude persisted for the 60-min test session. These results suggest that modulation of the startle reflex by CRH release at the level of the PnC, perhaps following activation of the amygdala.

THE ROLE OF ANGIOTENSIN II IN THE MOTIVATIONAL AND PREFERENCES DRINKING. L. Stolner-Weatherly*, J.N. Weatherly and W. Wright, Dept. of Psychology, Washington State University, Pullman, WA 99164-4820

While it is well established that intracerebroventricular (i.c.v.) infusion of Angiotensin II (AngII) induces a drinker preference for drinking different liquids (H2O versus a 16% sucrose solution) over a 24-hour period following an infusion of either AngII or aCSF. Male Sprague-Dawley rats were given an intake baseline for 3 hours through which AngII (100 pmol in 2 ul aCSF) or aCSF was delivered. After an infusion, an animal's fluid intake of either H2O, 16% H2O/sucrose solution, or 16% H2O/sucrose solution was measured. Thus, factors involved in AngII-induced drinking, such as whether drinking was motivated by a biological need for a fluid or whether fluid preference was controlled by a central mechanism could be measured. Furthermore, monitoring feeding over a 24-hour period allowed for the assessment of any interaction between these variables. Preliminary data suggests that an interaction exists between total fluid intake and fluid preference. Although AngII infusions mediate simple generalized drinking responses, it also appears that other motivational factors still exert control over an animal's drinking behavior.


The startle reflex is enhanced by both conditioned and unconditioned fear*. The amygdala appears to play a critical role in the mediation of these effects since lesions of various amygdala nuclei reduce startle amplitude, while electrical stimulation of the amygdala and its output pathways increase startle amplitudes (e.g. Davis, 1992). Several lines of evidence suggest that activation of cholecystokinin (CCK) receptors produces anxiety and panic in laboratory animals and humans. CCK is found in the basolateral nucleus of the amygdala. Systemic administration of CCK 1-4 or pentagastrin, which are selective for CCK A receptors, increases anxiety-like behaviors measured in a number of different experimental paradigms. Here we test whether the central infusion of CCK, either intracerebroventricularly (i.c.v.) or into the amygdala, increases startle amplitudes. Following i.c.v. infusion of the CCKBB agonist pentagastrin (0, 1.0, 10.0 mM in 5.0 ul), we recorded startle amplitudes for a period of 2 hours. No increases in startle amplitudes occurred over this period compared to pre-infusion baseline levels. In contrast, infusion of pentagastrin into the amygdala (0, 0.01, 0.1, 1.0, 10.0 mM in 5.0 ul) produced large increases in startle amplitudes at the three highest doses compared to pre-infusion baseline levels. These increases occurred 5-10 minutes following the drug infusion, and persisted for the remainder of the 30-minute test.

ROLE OF THE BED NUCLEUS OF THE STRIA TERMINALS AND AMYGDALA IN THE FACILITATORY EFFECTS OF CRH ON THE ACOUSTIC STARTLE REFLEX. Y. Lee* and M. Davis, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508

Corticotropin releasing hormone (CRH) is known to markedly increase the amplitude of the acoustic startle reflex after intraventricular (i.v.) infusion of CRH-enhanced startle; Swedlow et al. (1993). Intra-amygdala infusion of CRH did not mimic CRH-enhanced startle, we found that large electrolytic lesions of the amygdala complex blocked this effect. This suggested that the amygdala may play an obligatory role in the expression of this effect but may not be the only receptor system involved. We have investigated the role of the bed nucleus of the stria terminals (BNST) and the subnuclei of the amygdala in CRH-enhanced startle.

Electrolytic lesions, as well as NMDA lesions, of the BNST block CRH-enhanced startle. However, neither iontophoretic lesions of the central nucleus nor NMDA lesions of the basolateral nucleus of the BNST block CRH-enhanced startle, even though both amygdala lesions completely blocked fear-potentiated startle, another behavioral measurement sensitive to amygdala manipulation. These data suggest that the blockade seen with electrolytic lesions of the amygdala complex was due to damage of fibers of passage, perhaps afferent or efferent fibers of the BNST. At the present time, it is not clear whether the BNST is the primary receptor site which mediates CRH-enhanced startle, or whether it is an important relay station for expressing this effect. Currently we are testing both possibilities by infusing CRH directly into the BNST and investigating the level of various other structures which are close to the ventricles and projection to the BNST via the fornix, such as the ventral hippocampus, in CRH-enhanced startle.
665.1


Alcohol preferring AA rats, selectively bred for a high daily consumption of ethanol (EIOH > 5 g/kg, 10 % VAO) or DMSO over water, were tested with a variety of putative anticraving drugs, injected shortly before the 12 h test session. Absolute and relative (preference) EIOH intake, as well as ethanol and DMSO consumption were determined. In general, one drug could be categorized according to the particular profile of drug effect. One typical profile was obtained with (35)-flupenthixol, with a clear suppression of consummatory behavior (both food and fluid). Although EIOH intake was reduced by this compound, the effect was completely nonselective (consequently, EIOH preference was not affected). A different profile was obtained with imipramine and fenfluramine, as these compounds reduced both EIOH intake and preference. However, both compounds also reduced food intake and this effect was already present at doses below the doses which affected EIOH intake. The largest group contained compounds which were able to reduce EIOH intake and preference, but this effect occurred at similar doses which suppressed food intake (fluoxetine, lisdexamfetamine, naltrexone, pimozide) or pentylenetetrazol (PCP)-like drugs. Eight of the twelve reliably self-administered ethanol relative to water over a 3-hour period. Ethanol concentration (0.25 - 32 g/ml) was altered; increases in concentration produced a biconic preference in drug-reinforced responding relative to water and increases in ethanol intake. Individual differences in ethanol intake were considerable, and were representative of individual differences of other oral ethanol intake in rhesus monkeys. Naltrexone (0.032 - 0.32 mg/kg i.m.) reduced both ethanol and water responding across monkeys, and, in some cases, reduced ethanol-reinforced responding more than water responding. At doses that reduced ethanol-reinforced drinking, naltrexone had little or no effect on sucrose-reinforced drinking. This type of study in primates may be appropriate to begin to examine parallels to the studies in humans in which naltrexone decreases certain ethanol-related behaviors. (Supported by USPHS Grants DA 00254, DA 05325, DA 07268, and DA 08568).

665.2


The AA (Alko, Alcohol) rat line, selected for high alcohol drinking, has previously been shown to drink more water solution containing the opiate antagonists than its counterpart, the alcohol non-preferring ANA (Alko, Non-Alcohol) rat line (Hyttial and Sinclair, Psychopharmacology 111:409, 1993). The present experiments were conducted to see whether the relationship between ethanol and naltrexone intake by these lines would persist if an intravenous route of administration is used. Naive AA and ANA rats (6根本没有 per line) were trained to lever press using food as a reinforcer. Rats were then implanted with a subcutaneous cannula into the external jugular vein. After recovery, animals were trained to respond for a 0.1 ml of infusion of heroin (0.03 mg/kg/min) on a fixed-ratio (FR) 1 schedule with a 20-s epoch (TO) 3 sessions. One stable baseline was achieved, and significant differences were recorded in heroin m4 and 4 mg/kg/min. These findings do not provide evidence for a functional equivalence in the opiate self-administration but suggest that at least some factors directing the drug choice may be different when a nonoral route of administration is used. Supported by grants AA04859, AA04620, and AA07456.

665.3

NALTREXONE EFFECTS ON ORAL SUCROSE AND ETHANOL-REINFORCED RESPONDING IN RHESUS MONKEYS. E.D. Pakkarian, K.L. Williams and J.H. Wood. Departments of Pharmacology and Psychology, University of Michigan, Ann Arbor, MI 48109-0632.

Twelve rhesus monkeys were given opportunities to respond to obtain either sucrose (2.0 - 20 g/ml) or ethanol; most of the monkeys had prior experience of exposure to opiate drugs (naloxone, etorphine) or pentylenetetrazol (PCP)-like drugs. Eight of the twelve reliably self-administered ethanol relative to water over a 3-hour period. Ethanol concentration (0.25 - 32 g/ml) was altered; increases in concentration produced a biconic (Figure 1) preference in drug-reinforced responding relative to water and increases in ethanol intake. Individual differences in ethanol intake were considerable, and were representative of individual differences of other oral ethanol intake in rhesus monkeys. Naltrexone (0.032 - 0.32 mg/kg i.m.) reduced both ethanol and water responding across monkeys, and, in some cases, reduced ethanol-reinforced responding more than water responding. At doses that reduced ethanol-reinforced drinking, naltrexone had little or no effect on sucrose-reinforced drinking. This type of study in primates may be appropriate to begin to examine parallels to the studies in humans in which naltrexone decreases certain ethanol-related behaviors. (Supported by USPHS Grants DA 00254, DA 05325, DA 07268, and DA 08568).

665.4

GENETIC DIFFERENCES IN NALOXONE ENHANCEMENT OF ETHANOL-INDUCED CONDITIONED TASTE AVERSION. J. Broadbent, H.Y. Linder, G.L. Cunningham, Medical Psychology, Oregon Health Sciences University, Portland, OR 97201.

The influence of opioid systems on the aversive hedonic effects of ethanol was examined in alcohol-preferring (C57BL/6J) and avoiding (DBA/2J) strains of mice using the conditioned taste aversion paradigm. Naloxone (1 or 3 mg/kg) or ethanol (3 g/kg) given alone did not produce an aversion but a novel flavoring agent (fructose, 1.11:409, 1993). The effect of ethanol was due to a ‘floor’ effects on consumption. A lower dose of ethanol (1 g/kg) produced a modest taste aversion in DBA/2J mice that, again, was not potentiated by naloxone. Mice trained with high-dose conditioning with a high ethanol dose, however, significantly suppressed consumption indicating that naloxone’s failure to enhance the effects of ethanol was due to a floor effect. These results reveal a selective enhancement of the aversive effects of ethanol in C57BL/6J mice by naloxone, suggesting that genetically determined differences in endogenous opioid systems in alcohol-preferring mice may mitigate ethanol’s aversive effects. Supported by NIAAA grants AA08621 and AA07468.

665.5

NALTREXONE EFFECTS ON ACQUISITION AND REDUCTION OF ETHANOL CONSUMPTION IN C57BL/6J MICE. T.J. Phillips, C.M. Wenger, and J.D. Dow, V.A. Med. Ctr. and Dept. of Medical Psychology, OR Health Sci. Univ., Portland, OR 97201.

Opiate receptor antagonists, such as naloxone and naltrexone, have shown to reduce ethanol (ETOH) consumption in ETOH preferring rodent lines and strains. Most studies have concentrated on antagonist effects in animals that have already acquired high levels of ETOH drinking. We have performed three two-bottle choice drinking (water vs. 10% ETOH) using the ETOH preferring C57BL/6J mice. The first was a more recent findings of naltrexone’s effects on reducing established ETOH consumption. An increasing i.p. dosing regimen was used, and significant effects of naltrexone on ETOH preference and consumption were found at lower doses; these effects were dose dependent. The second study examined the effects of naltrexone on the acquisition of ETOH consumption. Time-release naltrexone pellets mice drank more ETOH and showed greater ETOH preference than did placebo pellets mice. The third study examined the effects of i.p. injected naltrexone on acquisition of ETOH drinking. Naltrexone injected mice consumed significantly more than placebo injected mice and did saline treated mice. There appear to be important consequences of consuming and possibly ETOH experience on behavioral responses of naltrexone on ETOH consumption. Supported by grants from the Dept. of Veterans Affairs and the Alcoholic Dev. Med. Res. Found.

665.6


Opioid peptides have been implicated in various behavioral actions of alcohol, including the reinforcing effects, however the role of specific brain sites remains to be explored. The present study examined the effects of intracebroventricular (ICV) or intracerebral (IC) injections of an opiate antagonist on ethanol self-administration. The nucleus accumbens and central nucleus of the amygdala were selected as IC sites given that these regions have been implicated in the behavioral effects of opiate drugs. Male Wistar rats were trained in a limited access paradigm (1-min sessions) to respond for ethanol (10% v/v) and water in a two-bottle choice condition using a saccharin fading procedure. Following the establishment of stable baseline responding for ethanol (± 20%), animals were implanted stereotaxically with a guide cannula above the lateral ventricle (ICV) or with bilateral guide cannulae either above the nucleus accumbens or amygdala. Following postoperative recovery of stable baseline responding, the rats were tested 15 min after ICV or IC microinjections of an opiate antagonist (naloxone - 10 µg) or saline (0 - 2µg). Methylenehaloxamine injections dose-dependently reduced ethanol self-administration. Injections into the amygdala significantly reduced responding for ethanol at doses of 0.25 - 0.50 µg, with higher dose administrations being required in the nucleus accumbens (0.50 - 1.0 µg) and still higher doses were needed ICV (1.0 - 2.0 µg). These results provide evidence that opioids, particularly in the amygdala, may reduce ethanol self-administration. Supported by grants AA08459, AA04620 and NIDA AA15043.
665.7 DECREASED VOLUNTARY ETHANOL CONSUMPTION IN TRANSGENIC MICE LACKING β-ENDORPHIN J.E. Grisel*, N.J. Graham*, J.K. Mogil, J.K. Belknap and M.J. Low Department of Psychology, Oregon State University, Corvallis, OR 97331.

The opioid peptide, β-endorphin, has been purported to play a role in alcoholism. Previous studies tested this idea in mice lacking β-endorphin (POMCX∗/- mice) due to transgenic alteration of its precursor, the proopiomelanocortin (POMC) gene. Introduction of a premature translational stop-codon into the POMC gene resulted in a truncated prohormone lacking the carboxyl-terminal region coding βendorphin, but normal expression and regulation of other peptide products of the POMC gene. We have previously demonstrated that POMCX∗/- mice display deficient opioid stress-induced analgesia, but are not impaired in their analgesic responses to exogenously administered opiates (Rubenstein et al., submitted). In the present study, these adult male POMCX∗/- mice and their wild-type littermates (129/6 hybrids) were given home-cage, two bottle choice access to either water and solutions containing either ethanol (7% vol/vol) or the selective µ-agonist, etonitazene (3mg/ml). POMCX∗/- mice demonstrated both a decreased preference for and consumption of ethanol compared to wild-type mice, but no other selfadministration were apparent. These data support the idea that β-endorphin plays a role in mediating ethanol consumption, and extend previous findings that mice lacking β-endorphin do not have altered opiate responsivity. Supported by grants from the NIH and the Markey Charitable Trust (MLJ), and a VA Merit Review (JKB).


A number of studies have indicated that the reinforcing effects of ethanol are mediated, at least in part, by its effects on the activity of the endogenous opioid system. Genetically determined differences in the activity of the endogenous opioid system, under basal conditions or following exposure to alcohol, may be important in controlling alcohol consumption. Indeed, previous studies have demonstrated a higher content of pro-opiomelanocortin (the precursor to beta-endorphin) mRNA in the arcuate nucleus of the AA than of the ANA rats. The objective of the present studies was to investigate whether the presence of differences in the contents of proenkephalin and prodynorphin mRNAs in distinct regions of the brain between AA and ANA rats under basal conditions, using 32P-labelled oligonucleotide probes for proenkephalin and prodynorphin and in situ hybridization techniques. Results indicated that in the arcuate nucleus and septum the content of proenkephalin mRNA was significantly higher in the AA than ANA rats. On the other hand a lower content of prodynorphin mRNA was observed in some brain regions of the AA than of the ANA rats. In conclusion, such differences in the content of mRNAs of the endogenous opioid peptides between the AA and ANA rats may be important in controlling voluntary alcohol consumption by these lines of rats. Supported by a grant from the Natural Science and Engineering Research Council of Canada.

665.11 INVOLVEMENT OF THE PEDUNCULOPONTINE NUCLEUS IN REGULATING ALCOHOL DRINKING. N. Kater, W. J. McIlrath, L. Lamming, T. K. IJ, M.J. Murphy*, Dept. of Psychology, Idaho State University, Pocatello, ID 83209, USA and Dept. of Psychol., Purdue Sch. of Sci., IUPUI, Indianapolis, IN 46202.

Experiments were conducted to determine if the pedunculopontine nucleus (PPN) is involved in mediating ethanol drinking behavior in the alcohol-preferring P line of rats. Female P rats were given limited access (24h) to 10% (v/v) ethanol and 0.0125g% (5mg/ml) saccharin solutions. During limited access, P rats consumed 10.4 ± 0.6 ml of saccharin and 1.6 ± 0.3 ml of ethanol. Food and water were available ad libitum. Cholinergic agents were microinjected unilaterally into the PPN immediately prior to ethanol access. Carbocaine (1-4μg/0.5μl), which inhibits cholinergic neuronal activity within the PPN, dose-dependently decreased ethanol intake within the first 30 min (70% decrease at the highest dose; p<0.05). However, by the end of the 2 hr period, the actions of carbocaine had begun to diminish with only the highest dose being effective. Carbocaine also dose-dependently reduced saccharin intake within the first 30 minutes (90% decrease at the highest dose; p<0.05). The results with carbocaine suggest that normal activity of the cholinergic PPN system is required for maintaining ethanol drinking behavior. Scopolamine (0.5-15μg/5μl), which stimulates cholinergic neuronal activity within the PPN, dose-dependently decreased ethanol intake within the first 30 min (65% decrease at the highest dose; p<0.05); ethanol consumption was partially recovered by the 2 hr period. On the other hand, microinjection of scopolamine into the PPN did not alter saccharin intake after 30 minutes. The findings with scopolamine suggest that increased activity of the cholinergic PPN system selectively reduces ethanol intake, possibly by mimicking the actions of alcohol itself and consequently requiring less ethanol consumption to produce the same CNS effect. Overall, these results support an involvement of the cholinergic PPN in regulating alcohol drinking behavior of the P line of rats. (supported in part by AA 0855, AA 07462, AA 0761).

We studied the effects of α-MSH on the acquisition of a preference for ethanol. Thirty-six Sprague-Dawley male rats had access to a 3% v/v solution of ethanol for the first 6 days, followed by six days of 6% v/v solution of ethanol and six days of 9% v/v solution of ethanol in a limited access paradigm. The solutions of ethanol were available for one hour a day starting at 1600h, and intake of ethanol and water were measured daily prior to the presentation of the solutions of ethanol. Rats were injected IP with either 0.001, 0.003, 0.006, or 0.1 mg α-MSH 20 minutes before the presentation of the solution of ethanol. A 6 X 3 X 6 (dose X concentration X days) mixed analysis of variance, with concentration and days as the repeated measures, yielded a significant two-way (dose X ethanol) interaction, p < 0.05. All doses of α-MSH increased intake of ethanol as the concentration of ethanol increased except the 1 mg/kg dose of α-MSH which decreased intake of ethanol as the concentration of ethanol increased. A significant main effect for ethanol was obtained, p < 0.05, with a higher overall intake of ethanol for the 6% and 12% v/v solutions of ethanol. There was also a significant main effect for dose, p < 0.05. An overall trend analysis on the doses of α-MSH yielded a significant quadratic trend, p < 0.05, indicating an inverted U shaped dose-response relationship among the doses of α-MSH. These results suggest that α-MSH influences the acquisition of a preference for ethanol in rats.

665.15 NITRIC OXIDE SYNTHASE INHIBITION REDUCES ALCOHOL WITHDRAWAL HYPERMATURITY, ALCOHOL PREFERENCE AND CORTICAL HYPERMATURE AFTER ACUTE ALCOHOL EXPOSURE. P. De Mitri*, G. W. Pennypacker and F. Lallendorf. Lab. Psychobiology, Univ. of Louvain, Belgium.

Nitric oxide (NO) modulates the vascular system and also interacts with alcohol. L-NNA, a selective NO synthase inhibitor, was mixed with water in the drinking bottle at 500 mg/kg/day during postnatal chronic alcoholization. Rats were kept for 21 days in the alcoholization chamber before recording of 1) the motility during the withdrawal syndrome 2) the preference for ethanol in a free choice way versus 10% (v/v) ethanol solutions and finally 3) the microvascular morphometric quantitation of the vessels length in the fronto-parietal cortex. Results showed that 1) the hypermotility during withdrawal syndrome was significantly decreased in L-NNA rats 2) the alcohol preference cut-off, i.e. the moment when rats failed to drink preferentially ethanol, was significantly decreased in the L-NNA treated rats, while no difference occurred in the global liquid consumption between treated and untreated rats and 3) the hypermotility observed in the cortex was observed after chronic alcoholization was significantly decreased in L-NNA treated rats. L-NNA, a NO synthase inhibitor, reduced thus the behavioral dependence after alcoholization, the behavioral preference for alcohol as well as the microvascular length of the cortex supporting thus the direct implication of NO in alcohol abuse and its withdrawal.

665.17 RELATIONSHIP BETWEEN SPONTANEOUS SEIZURE OCCURRENCE AND SALICYLATE SPIN-TRAP ADDUCTS FROM_WEB THAT RAL BRAIN HOMOGENSES AFTER SINGLE AND REPEATED ETHOH DEPENDENCE EPISODES. M. Valletti* D.Y. Gavrin, W.W. Beauty, T. Tabakabatie & R.C. Floyd*, Division of Neurology, Department of Neurology, University of Oklahoma Health Sciences Center, Dept. of Free Radical Biology & Aging, Oklahoma Medical Research Foundation*, Oklahoma City, OK 73190.

ETHOH exposure increases free-radical generation, lipid peroxidation (LP), and (GSSG) with concomitant decreases in (GSH) and (GSH) in rat brain. The increase in LP is independent of acetadecyle production by both alcohol and LP levels of CNS hydroxy-radical species in rats exposed to either single or multiple (11) cycles of ethanol vapor exposure. The results of the oxidation of salicylate hydroxyl products (catechol, 2,3-Dihydroxybenzoate (2,3 DDB), and 2,3 Dihydroxybenzoate (2.5 DBBA)) were used to quantify levels of brain homogenate hydroxy-radical content and be used to observe the absence or presence of ethanol withdrawal. The present studies, we investigated the efficacy of nitrendipine and nimodipine, calcium channel blockers, to modify an EW symptom in a animal model of anxiety: male rat behavior in elevated plus-maze (EPM). Long-Evans hooded male rats were housed five rats/90 cm (air conditioned 6% v/v CO2). Rats were sacrificed 24h after the single exposure of 24 h or multiple exposures of 12 h each. Water control and ethanol treated rats (terminal BACs of 2-300 mg/dl for 24 h) were injected with 100 mg/kg salicylate, a trapping agent, one hour before rapid decapitation, tissue harvesting, and liquid nitrogen tissue immersion. Significant group differences (F(1,22)=22.7, p<0.01) were found in 2,3 DHBAs after ethanol withdrawal. Group significant-group differences were also found for both 2,5 DHBAs and the total DHBAs salicylate ratio after both single and multiple exposures. Positive correlations between the occurrence of spontaneous withdrawal seizures and the concentration levels of 2,3 DDBA and the total DHBAs salicylate was 0.82 (r=0.87, p<0.001) and 0.75 (r=0.59, p<0.001), respectively.

665.18 CALCIUM CHANNEL BLOCKERS REDUCE ETHALIN WITHDRAWAL-INDUCED ANXIETY IN MALE RATS. C.J. Wallis*, and H.L. Lal. Department of Pharmacology and SANT, University of North Texas Health Science Center, Fort Worth, TX 76107.

Ethanol modifies the activity of a variety of ligand-gated and voltage regulated ion channels. If increased calcium channel activity contributes to the ethanol withdrawal (EDW) syndrome, then reducing calcium channel activity during EDW should ameliorate some of the symptoms of ethanol withdrawal. In the present studies, we investigated the efficacy of nitrendipine and nimodipine, calcium channel blockers, to modify an EW symptom in a animal model of anxiety: male rat behavior in elevated plus-maze (EPM). Long-Evans hooded male rats were housed five rats/90 cm (air conditioned 6% v/v CO2). Rats were sacrificed 24h after the single exposure of 24 h or multiple exposures of 12 h each. Water control and ethanol treated rats (terminal BACs of 2-300 mg/dl for 24 h) were injected with 100 mg/kg salicylate, a trapping agent, one hour before rapid decapitation, tissue harvesting, and liquid nitrogen tissue immersion. Significant group differences (F(1,22)=22.7, p<0.01) were found in 2,3 DHBAs after ethanol withdrawal. Group significant-group differences were also found for both 2,5 DHBAs and the total DHBAs salicylate ratio after both single and multiple exposures. Positive correlations between the occurrence of spontaneous withdrawal seizures and the concentration levels of 2,3 DDBA and the total DHBAs salicylate was 0.82 (r=0.87, p<0.001) and 0.75 (r=0.59, p<0.001), respectively.
665.19


Department of Psychology, Kansas State University, Manhattan, KS 66506-3502. University of North Carolina School of Medicine, Chapel Hill, NC 27559-7178.

Rats selectively bred for high (HI) and low (LO) alcohol preference were used to examine the acquisition and extinction of an alcohol-place aversion. Each animal was given one pair of 6% (v/v) alcohol solution followed by intubation of a 0.15 M LiCl solution. On the day following the acquisition trial, animals were tested for taste reactivity to 6% alcohol. The animals then received seven extinction trials with the alcohol solution, each trial given every other day. Analysis of the taste reactivity data showed that HI rats produced significantly more ingestive responses and fewer aversive responses than LO rats. Both HI and LO rats showed alcohol avoidance on the initial extinction test, the HI rats continued to show avoidance over the course of the seven extinction trials. These results suggest that, while HI rats acquired an avoidance of alcohol when it was paired with illness, the avoidance did not appear to entail a shift in palatability nor was it as durable as that found in ANA rats.

665.20

REPEATED EPISODES OF ETHANOL WITHDRAWAL LOWER PENTYLENETETRAZOL (PTZ) SEIZURE THRESHOLD DOSE IN MICE. J. L. Shay-Graham*, R. H. Resch, K. G. Fernandes, and D. O. Becker.

Med. Univ. of S. Carolina and VAMC, Charleston, S.C. 29426.

We have established a model in which multiple episodes of EtOH withdrawal result in an exacerbation of handling-induced convulsions (HIC) in adult C3H mice compared to mice that have been withdrawn a single time. The present experiment was designed to examine whether repeated EtOH withdrawals alters sensitivity to the proconvulsant effects of the GABAa receptor antagonist PTZ, as assessed by the tail vein infusion method. Male mice were divided into six treatment groups: a multiple withdrawal (MW) group received 4 cycles of 16 hr EtOH vapor separated by 8 hr periods of abstinence; a single withdrawal (SW) group received a single 16 hr bout of EtOH; and controls (C) received no EtOH. At withdrawal, blood ethanol levels did not differ among the EtOH withdrawal-induced animal groups, but EtOH was determined to be in the MW group as compared to SW and C groups (P < 0.001). Separately, animals were administered PTZ (4 mg/kg) i.v. either 8 or 24 hr post-withdrawal. At the 8 hr timepoints, threshold doses for tonic-clonic (TC) and tonic hindlimb extension (THE) were significantly lower in the MW group as compared to the SW and C groups (TC: 17.9 ± 1.3; 40.5 ± 7.7; 38.6 ± 7.2 mg/kg, respectively; THE: 20.5 ± 1.3; 50.9 ± 10.8; 80.7 ± 11.8 mg/kg, respectively). In addition, these treatment groups differed in the development of the seizure where the time between the myoclonic jerk (A) and TC was significantly less in the MW as compared to SW and C groups (P < 0.01). At 24 hr timepoint, preliminary results indicate a lower seizure threshold dose to MU in the MW group. The latter endpoints are currently being evaluated. These results suggest that modifications at the GABAa receptor complex may underlie the potentiated seizure activity resulting from repeated ethanol withdrawals. Supported by NIAAA and VAMC.

666.1

ALCOHOL-ILLNESS ASSOCIATIONS IN THE SELECTIVELY BREED AA AND ANA RATS. Richard L. Elder*, Illinois State University, Campus Box 4620, Normal, IL 61790-4620 and Nancy Badia Elder, and Stephen W. Kiefer, Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Selectively bred AA and ANA rats were used to examine the acquisition and extinction of an alcohol-illness association. Each animal was given one prioring of a 6% (v/v) alcohol solution followed by intubation of a 0.15 M LiCl solution. On the day following the acquisition trial, animals were tested for taste reactivity to 6% alcohol. The animals then received seven extinction trials with the alcohol solution, each trial given every other day. Analysis of the taste reactivity data showed that AA rats produced significantly more ingestive responses and fewer aversive responses than ANA rats. Although both AA and ANA rats showed alcohol avoidance on the initial extinction test, the AA rats continued significantly more alcohol over the course of the seven extinction trials. These results suggest that, while AA rats acquired an avoidance of alcohol when it was paired with illness, the avoidance did not appear to entail a shift in palatability nor was it as durable as that found in ANA rats.

666.2

TASTE REACTIVITY TO ALCOHOL IN RATS SELECTIVELY BREED FOR SENSITIVITY TO A SEROTONIN-1A AGONIST. M. E. Badia-Elder*, S. W. Kiefer, D. H. Overstreet, and A. H. Rozvani.

Kansas State University, Manhattan, KS 66506-3302. University of North Carolina School of Medicine, Chapel Hill, NC 27559-7178.

Rats selectively bred for high (HI) and low (LO) hypothermic responses to the serotonin-1A agonist 8-hydroxy-(2-amino)-tetralin (8-OH-DPAT) were tested for taste reactivity to five concentrations of alcohol (5%, 10%, 20%, 30%, and 40%), sucrose, quinine, and water both prior to and following 21 days of two-bottle consumption testing with 10% alcohol and water. Both groups showed similar increases in ingestive and decreases in aversive responding to alcohol from the first to second set of taste reactivity tests. However, HI and LO rats did not differ in taste reactivity to alcohol. During consumption testing, HI rats consumed only slightly more alcohol than LO rats. The results indicated that HI and LO rats do not differ greatly in alcohol reactivity or consumption. Both groups showed significant increases in ingestive and decreases in aversive responding to alcohol, but HI rats consumed more alcohol than LO rats. The results indicated that HI and LO rats do not differ greatly in alcohol reactivity or consumption. During consumption testing, HI rats consumed only slightly more alcohol than LO rats. The results indicated that HI and LO rats do not differ greatly in alcohol reactivity or consumption.
666.3
Quantitative Trait Loci analyses suggest that the 5HT1b serotonin receptor subtype may be a candidate gene that influences several ethanol-related traits. Null mutant mice have been developed that lack the gene coding for the 5HT1b receptor. Pharmacological evidence suggests that these mice might show reduced sensitivity to several ethanol effects. Fischer inbred genetic lines differ in 5HT1b receptor expression and ethanol-induced hypothermia and tolerance. (-)- and (+)-mice had diminished response to an acute 3 g/kg EtOH injection as compared with (+/-) mice. With 3 daily injections, tolerance developed in all genotypes to an equivalent extent. Because the hypothermia data suggested partial loss of response in heterozygotes even though they thought to have normal 5HT1b receptor levels, wild-type and additional animals 10 mg/kg RU 24969, a 5HT1b receptor antagonist (+/-) animals had a large, and (+) mice a very small hypothermic response: heterozygotes had an intermediate one. A week later, these mice were tested for their acute withdrawal response following a single 4 g/kg dose of EtOH, but the three genotypes did not differ. Other effects of EtOH were also tested. These studies are consistent with a role for serotoninergic systems in modulating responsiveness to some, but not all, effects of EtOH. Support by NIAAA, NIDA, & Dept. of Veterans Affairs.

666.5
ETHANOL- AND NICOTINE-PREFERING TRANSGENIC MICE DISPLAY REDUCED SEROTONIN CONTENT IN NUCLEUS ACCUMBENS. R.W. Steiger*, C. Skoga, M.T. Fadden and A. Bartke. Dept. of Physiology, SIU School of Medicine, Carbondale, IL 62901.
Male transgenic (T) mice overexpressing the bovine growth hormone gene more and exhibit greater preferences for ethanol (EtOH) and nicotine solutions than non-transgenic litter mate controls (Pharmacol. Biochem. Behav. 61:503, 2001). Since mesolimbic brain monoamines may modulate drug-induced reinforcement, we examined indices of content and turnover of serotonin (5-HT) and dopamine (DA). In vivo microdialysis of male T and non-T mice, one hour after i.p. injection of either saline or 1.5 g/kg EtOH. Results showed that 5-HT content in nucleus accumbens (Nacc), but not corpus striatum was lower in T than in non-T mice (1.7 +/- 0.5 vs. 4.6 +/- 1.0 ng/mg tissue, P < 0.01), one hour after i.p. saline. In contrast, one hour after i.p. EtOH, T and non-T mice did not differ in Nacc or corpus striatum after saline or EtOH. Reduced 5-HT function in reward-relevant brain regions may be associated with increased vulnerability to drug-induced reinforcement. (Supported by NIAAA Grant 1-R03-AA09457.)

666.6
DIFFERENTIAL IMPACT OF ETHANOL ON EXTRACELLULAR LEVELS OF SEROTONIN AND GLUTAMATE: A COMPARATIVE STUDY IN LEWIS AND FISCHER 344 RAT STRAINS. M. Castanon, C.L. Nefcy, J. Fuchs, S. Bielawska, B. Blocker, H. Grossman, B. Sakmann, Dept. of Med., Deps. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, 116142, West Haven, CT 06516.
The present study investigated the impact of systemic ethanol administration on extracellular levels of 5-HT and Glutamate (Glu) in the prefrontal cortex (PFC) and the nucleus accumbens (NA) in Lewis (LEW) and Fischer (FIS) inbred rats using microdialysis in awake animals. We chose to compare basal EtOH levels in the two rat strains, given our previous findings that EtOH levels were greatly elevated in LEW vs. FIS rats and are thought to be genetically predisposed to drug abuse. The extracellular levels of 5-HT and Glu in the PFC of either strain were not significantly altered by i.p. ethanol at 0.5, 1.0 or 2.0 g/kg; all p values > 0.05. Similarly, injections of ethanol at 0.5 or 2.0 g/kg did not result in any significant change (p>0.05) in NA dialysate concentrations of 5-HT or Glu in either strain. At 1 g/kg, ethanol caused a 44%±16 increase in NA-dialysate 5-HT levels in LEW rats (p=0.006). Again, no significant change was observed in F344 rats (p=0.66). Preliminary data indicate that ethanol (1g/kg) causes a non-significant trend toward an increase in extracellular Glu in NA in both strains. Basal levels of Glu in both the NA and PFC of LEW rats were significantly lower than in F344 rats (p=0.05 and 0.04, respectively). Basal levels of 5-HT were also lower in LEW rats, but the levels of extracellular significance were marginal (p=0.10 and 0.08). These findings indicate that responsiveness of 5-HT to ethanol, or basal levels of Glu may be important in strain differences between F344 and Lewis rats in response to acute ethanol administration. Supported by DA 08073, DA 08227, The Yale VA Alcoholism Research Center, and the West Haven VA Center for the study of PTSD.

666.7
ETHANOL EFFECTS ON EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS: COMPARISON BETWEEN FISCHER AND LEWIS RAT STRAINS. Z. Moczydlowski, J.W. Blakely. Yale Univ. Sch. of Med., Deps. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, West Haven, CT 06516.
Increasing attention has focused upon comparisons between strains of animals with differing propensities to consume alcohol and other drugs of abuse in order to gain insight into potential biochemical bases for drug abuse vulnerabilities. This includes the Fischer and Lewis rat strains, which have large differences in self-administration of alcohol, cocaine and opioids. The neurochemical basis of ethanol reward is unclear, without the same dependence upon intake dopamine (DA) innervation of the ventral tegmental area (VTA) is seen with psychostimulants. We have made a microdialysis comparison of the effects of ethanol upon both extracellular DA and serotonin (serotonin-containing) in awake animals of the Fischer and Lewis rat strains. In the Fischer strain, there was no significant increase in DA in either strain at 0.5 g/kg (p<.05). At 1.0 g/kg, a dose characteristic of ethanol use in primates, a significant increase in DA was noted in the Fischer strain (180 % of baseline at 60 min post-ethanol), with a significant difference in response between strains (p=0.02) in animals with repeated measures (ANOVA). The 2.0 g/kg dose caused a nearly significant increase in the Fischer strain, no significant increase in the Lewis, and no significant difference in responsiveness between the two strains. Basal ethanol levels in the two strains were not different. Our results are not consistent with an elevated mesolimbic DA response to ethanol as a basis for the enhanced preference for Lewis rats for ethanol. Supported by DA 08073, DA 08227, DA 04000, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CBW.

666.8
ALCOHOL SELF-ADMINISTRATION AND AGGRESSION IN RATS: DOPAMINE AND SEROTONIN IN N. ACCUMBENS. A. Kam, J. van Erp and K.A. Mouw. Department of Psychology, Tulah University, Medellin, CO 00215.
Ethanol, when self-administered orally, enhances aggression in a subpopulation of resident rats confronting an intruder. These resident rats, characterized neurochemically, using in vivo microdialysis of dopamine and serotonin in the n. accumbens, Male Long-Evans rats, housed with a female, smaller intruder in their home cage during 5 min tests. Rats which consistently attack intruder were trained to drink a 10% ethanol solution during 15 min access in their home cage, using a sucrose-fading technique. After ethanol intake stabilised, intruder tests were conducted 2/wk, starting 5 min after the ethanol session. Blood samples were taken directly after the fight from the orbital sinus, under isoflurane anesthesia. Under these conditions, rats drank up to 1.0 g/kg, resulting in BAC levels in a range of 10-80 mg/dL, but mostly 20-40 mg/dL. Aggressive behavior after ethanol self-administration was enhanced in some animals (n=5) and unchanged in others (n=3), similar to previously reported results from tests with experimentally administered ethanol. In a second experiment changes in catecholamines in the nucleus accumbens were monitored during ethanol drinking and subsequent intruder tests. Ten min samples were taken during 60 min baseline, 10 min ethanol exposure, 10 min intruder test and 120 min recovery. During the intruder tests rats displayed the classic behavior of aggression, displaying disturbances, and responding vigorously to the intruder by attacking, threatening and biting. Without ethanol, resident rats displayed the classic behavior of aggression, displaying disturbances, and responding vigorously to the intruder by attacking, threatening and biting. We found no significant differences in the catecholamines even after ethanol. The results support the hypothesis that ethanol self-administration and the subsequent intruder confrontation: no change or even a decrease in dopamine is observed during and after intruder confrontations without ethanol. Preliminary results suggest that serotonin levels and perhaps changes in the microdialysis measurements are evaluated in relation to ethanol's aggression enhancing effects.
COMPARISON OF THE EFFECTS OF INTRAVENTRICAL, INTRA-NEOSTRIATUM, AND DIRECTLY PERFUSED ETHANOL ON EXTRACELLULAR DOPAMINE LEVELS IN NUCLEUS ACCUMBENS OF LEWIS RATS. J. Chen*, J. Li, and E.L. Gardner, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, New York, NY 10461.

Enhancement of synaptic activity in the nucleus accumbens (Acc) terminal projections of the mesolimbic dopamine (DA) system is one of the few neurochemical changes associated with drug of abuse (Chen, Sem Neuropsychopharmacol 5:315-320, 1993), and drug action on this system is hypothesized to be an important component of its function (Wise, Pharmacol Biochem Behav 13[4]:213-223, 1980). W. W. Bosco, and Brain Res Bull 12:203-208, 1984). However, ethanol's actions on these brain systems are less clear. Even in the different routes of ethanol administration on extracellular Acc:DA levels remains unclear, as does interaction between routes of administration and self-administration or exogenous administration. We studied the effect of three routes of ethanol administration on behavior and Acc:DA overflow (by in vivo microdialysis) in ethanol-prefering Lewis rats. Intraperitoneal (IP) ethanol injection (0.5, 1.0, and 2.0 g/kg) and intragastric ethanol gavage (0.5, 1.0, 2.5, and 10.0 g/kg) produced significant motor effects (ataxia, etc.), but direct Acc:DA perfusion (0.01, 0.1, 1.0, and 5.0% v/v) did not. Acc:DA did not change, but intragastric ethanol gavage did not. Intragastric ethanol consumption may require volitional self-administration to activate Acc reward system (Moolton & Kornetsky, Alcohol 7:221-225, 1990). (Supported by NIAAA grant AA0547, NIDA grant DA10622, and the Aaron Diamond Foundation).

TIME-DEPENDENT EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. D. Lyons*, M.D. Miller, A.M. Craig, A. A. Hedgecock, S. L. Hart, and L. A. Porrino, Dept of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Medical Center Blvd., Winston-Salem, NC 27157.

The present study tested the hypothesis that the effects of ethanol administration on brain activity depend upon the length of time following administration. A number of reports, for example, support the assertion that the rewarding effects of alcohol intake coincide with the ascending limb of the blood alcohol curve (BAC) and the depressant effects coincide with the descending limb. To understand better the neurobiology of ethanol intake, we evaluated the functional consequences of acute administration in awake rats at 5 and 15 minutes after treatment (1.0 g/kg ethanol or saline, ip), using the quantitative [14C]iodoamphetamine method for measurement of regional cerebral blood flow. These time-points were chosen because, although the 5 min point is on the ascending limb of the BAC, the same blood ethanol levels exist at each point, thus reducing potential differences related to the bioavailability of ethanol. Findings indicate that blood flow is increased at 15 min time-point, which is within the olfactory tubercle, basolateral amygdala, and thalamus, and decreased at 15 min time-point. Blood flow was altered at 15 min, however, in the caudate, nucleus accumbens and hippocampus. Other brain regions were unaffected by treatment. These data are consistent with work from this laboratory measuring the effects of ethanol on cerebral metabolism and demonstrate that the pattern of brain activity is different on the ascending and descending limbs of the BAC despite similar blood levels of ethanol. (Supported by NIAAA grant AA02991).
666.15

EFFECTS OF CHRONIC ALCOHOL CONSUMPTION AND AGING ON DOPAMINE, SEROTONIN AND METABOLITE LEVELS.


The present study investigated the combined effects of chronic alcohol consumption and aging on the concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). The rats were of both significant age- and alcohol-related changes in mesocorticolimbic brain areas. There was a significant age-associated decline of dopamine, and an alcohol-associated reduction of 5-HT and 5-HIAA in the ventral tegmental area. These results are consistent with the recent findings of others reporting decreases in dopamine and serotonin levels with age and chronic alcohol consumption. Thus, it was suggested that they might respond differently to agents interacting with neurotransmitter systems known to modulate alcohol intake. Adult male FH, P, and AA rats were observed in their respective breeding colonies in Chapel Hill, Indianapolis, and Helsinki and housed individually in wire mesh cages under standard laboratory conditions. The rats had free access to food, water, and alcohol (10%, v/v). After establishment of stable baselines, rats were injected subcutaneously with either the dopamine agonist bromocriptine (1 mg/kg), the serotonin-1A agonist 5-HT-DPAT (0.125 mg/kg), the serotonin releaser fenfluramine (2.5-10 mg/kg), the opiate antagonist naltrexone (3-5 mg/kg), or vehicle at 4-day intervals. The P rats exhibited a significantly greater suppression of alcohol intake after bromocriptine, while the FH rats exhibited a significantly greater suppression after 5-HT-DPAT. In contrast, the AA rats were comparatively resistant to each compound. These findings indicate that alcohol-prefering rat strains are differentially sensitive to several drugs which suppress alcohol intake, making it unlikely that a single pharmacological treatment will be beneficial in all human alcoholics.

This research was supported by a grant from the USPHS AA08451-04A. Woods is the recipient of a USPHS fellowship - F31 AA05378.

666.16

STRAIN-DEPENDENT SUPPRESSANT EFFECTS OF DOPAMINERGIC, SEROTONINERGIC AND OPIOIDERIC AGENTS ON ALCOHOL INTAKE.

D.H. Overton, A.A. Rezvani, and A.B. Kampov-Polevoy, Skidmore Bowles Center for Alcohol Studies, UNC, Chapel Hill, NC 27599-7178.

Several strains of rats are known to consume large amounts of alcohol voluntarily, including the Fawn-Hooded (FH) rats and the selectively bred alcohol-prefering (P) and alcohol-accepting (AA) rats. Neurochemical differences have been reported in the rats, so it was suggested that they might respond differently to agents interacting with neurotransmitter systems known to modulate alcohol intake. Adult male FH, P, and AA rats were observed in their respective breeding colonies in Chapel Hill, Indianapolis, and Helsinki and housed individually in wire mesh cages under standard laboratory conditions. The rats had free access to food, water, and alcohol (10%, v/v). After establishment of stable baselines, rats were injected subcutaneously with either the dopamine agonist bromocriptine (1 mg/kg), the serotonin-1A agonist 5-HT-DPAT (0.125 mg/kg), the serotonin releaser fenfluramine (2.5-10 mg/kg), the opiate antagonist naltrexone (3-5 mg/kg), or vehicle at 4-day intervals. The P rats exhibited a significantly greater suppression of alcohol intake after bromocriptine, while the FH rats exhibited a significantly greater suppression after 5-HT-DPAT. In contrast, the AA rats were comparatively resistant to each compound. These findings indicate that alcohol-prefering rat strains are differentially sensitive to several drugs which suppress alcohol intake, making it unlikely that a single pharmacological treatment will be beneficial in all human alcoholics.

666.17

ETHANOL-INDUCED CHANGES IN ACTIVITY OF ALCOHOL-PREFERRING (P) AND NONPREFERENCE (NP) RATS: EFFECTS OF DOPAMINE (DA) AND 5-HT RECEPTOR ANTAGONISTS.


P rats, in comparison to NP and untrained randomly bred rats, orally self-administer ethanol (E) at high concentrations (+10%) and increase locomotor activity after parenteral and oral E. The present study examined the effects of intraperitoneal (IP) E on open field activity in both P and NP rats. Animals were injected with saline or E (0.25-1.0 g/kg) and open field (Digiscan) activity was measured at 10 and 60 min. Animals then were injected with the DA antagonist haloperidol (0.1-0.5 mg/kg, i.p.), the 5-HT antagonist MDL72222 (0.5-2.0 mg/kg, i.p), or saline prior to measurement of activity. NP rats showed lower activity at all E doses. P rats showed increased activity after low doses of E at 10 min. and depression at higher doses. Both pimozide and MDL72222 blocked the increase in activity in P rats. Both antagonists increased the depressant effects of E in both lines. These data support the putative role of dopamine and 5-HT receptor mechanisms in effects on activity (Supported in part by AA06263 & RR08016 and Temple University).

666.18

INCREASES IN VOLUNTARY ALCOHOL CONSUMPTION IN RATS FOLLOWING A SINGLE LARGE DOSE OF DMI.

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In the course of a series of studies to examine the relationship between lesions of the prefrontal cortex (PFC) in Wistar rats and voluntary alcohol consumption, we report the following phenomenon. Male Wistar rats were subjected to bilateral aspiration lesions of the PFC, injections of 5-7-dihydroxytryptamine (5,7-DHT) or sham lesions. The 5,7-DHT treatment required pre-treatment with 25 mg/kg p.o. desmethyl-IMIPRAMINE (DMI), a catecholamine uptake blocker that is commonly given to enhance the 5,7-DHT exposure to serotonergic neurons. The controls used in the lesion group were not given DMI. All lesions were given during deep anesthesia induced by 65 mg/kg p.o. pentobarbital. After the lesions, rats were placed on a 6-week, modified sucrose-fading procedure during which they were exposed to increasing concentrations of alcohol-containing solutions. At each concentration of sucrose (Suc) and ethanol (ETOH) including 10% Suc:35% ETOH, 15% Suc: 5% ETOH, 3% Suc:5% ETOH and 5% Suc:10% ETOH the control rats that had had lesions did not respond to alcohol in the control group without DMI. The increased drinking was not generalized since the DMI group drank less than the controls without DMI when a 20% sucrose alone solution was tested. The rats had food and tap water available at all times. An unusual aspect of the finding is that the increased consumption of ethanol solutions lasted throughout the length of the study. Following the final solution presentation, all animals were decapitated and the brains rapidly dissected. Brain regions are analyzed for norepinephrine, dopamine and serotonin and their metabolites to determine whether the single dose of DMI produced a measurable change in transmitter level (supported by NIAAA P30 AA11119).
INVolvement of central, but not peripheral, NICOTINIC ACeTylCholine reCePTorS in the DOpAmine ACTIVATING and reINFORCing EFFectS of EthANOL: O. Blomgren*, J.A. Enged and B. Sörderpalm, Inst. of Physiology and Pharmacology, Dept. of Pharmacology, Göteborg University, Medicinsparken 7-5, 413 45 Göteborg, Sweden.

We have previously shown that mecamylamine (10 mg/kg i.p.), a blood brain barrier penetrating antagonist at the nicotinic acetylcholine receptor (nACHR), completely blocks ethanol-induced (2.5 g/kg i.p.) dopamine (DA) overflow in the nucleus accumbens in Wistar rats, as measured by in vivo microdialysis. Mecamylamine also decreased voluntary ethanol intake in high preferring but not low preferring rats, classified after a pre-experimental screening period in a free choice situation between an ethanol solution (6%) and water. Hence, we hypothesized that ethanol-induced DA release, which may be of importance for its reinforcing effect, is mediated via activation of central nACHR, perhaps through a direct ethanol-nACHR interaction. Involvements of peripheral nACHR could, however, not be excluded.

In the present study, hexamethonium, a nACHR antagonist which does not penetrate the blood brain barrier was used in order to elucidate the possible involvement of peripheral nACHR in the previous findings. Hexamethonium-chloride was administered (10 mg/kg i.p.) 20 minutes prior to ethanol (2.5 g/kg i.p.), and DA overflow in the rat nucleus accumbens was measured by in vivo microdialysis. Although dialysis probe insertion might cause a discrete blood brain barrier defect resulting in hexamethonium diffusion into the brain, no antagonism of ethanol-induced DA overflow was observed. Furthermore, hexamethonium treatment did not alter ethanol drinking behavior in high preferring or low preferring rats. In conclusion, unless mecamylamine and hexamethonium display significant pharmacodynamic differences in addition to their pharmacokinetic differences, peripheral nACHR are not likely involved in the DA activating and reinforcing effects of ethanol.

667.1

Glutaminergic dysfunction has long been implicated in psychosis. Based on this consideration, the present study of antipsychotic drug interactions with the NMDA receptor was undertaken. In cortical tissue extensively washed to reduce residual levels of glutamic and glycine, [3H]MK-801 binding is minimal. However, addition of either ligand at non-saturating concentrations increases ionophore opening resulting in a two- to threefold enhancement of [3H]MK-801 binding and is thus commonly used as a functional assay of NMDA activity. Addition of either chlorpromazine, haloperidol or clozapine all further increased the already elevated [3H]MK-801 binding caused by either glutamate and/or glycine. This effect was prominent at drug concentrations similar to that found in schizophrenic patients' plasma water or CSF when antipsychotic efficacy is achieved. At higher concentrations inhibition of NMDA activity was seen for all drugs. Augmentation at low and inhibition at higher concentrations was confirmed in electrophysiological studies of glutamatergic transmission in the striatal slice-preparation. These data, consistent with partial agonism, suggest that a unique action at glutamate synapses contributes to antipsychotic efficacy and further implicate glutamatergic dysfunction in the etiology of schizophrenia.

667.3

The effects of clozapine and haloperidol on extracellular levels of dopamine were examined in cortical and subcortical regions of the rhesus monkey brain using intracranial microdialysis. MRI-directed stereotaxic surgery was used to implant guide cannulae in three young adult male animals. Microdialysis probes were lowered into discrete brain regions through the guide cannulae while animals were under isoflurane gas anesthesia. Several dopamine-terminal-containing cortical regions were examined. These include: dorsolateral prefrontal cortex (Brodmann's area 46), medial prefrontal cortex (areas 8 and 9), and premotor cortex (area 6). Subcortical regions examined include the caudate and putamen. Basal extracellular levels of dopamine were reliably measured in all of these discrete brain regions. A stable baseline in extracellular dopamine was established for a minimum of one hour before drug administration. Clozapine (2 mg/kg i.v.) produced a significant increase in extracellular levels of dopamine in all brain regions examined, both cortical and subcortical. Haloperidol (0.2 mg/kg i.v.), in contrast, produced little or no change in extracellular dopamine levels in cortical regions or in the caudate. These results provide evidence in the non-human primate that acute administration of the atypical antipsychotic drug clozapine exerts a potent effect on dopamine that is comparable to the typical drug haloperidol. Supported in part by USPHS Award MH-44866.

667.4
MODIFICATION OF CLOzapINE-ELICITEd Fos EXPRESSION in the PreFRONTAL CORTEX AFTER BASOLATERAL AmYGDAla LEsIONS. D. S. Cameron, C. D. Young and A. Y. Deutch, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508 and the VA Medical Center, West Haven, CT 06516.

Administration to rats of the atypical antipsychotic drug clozapine results in a marked increase in the expression of the immediate-early gene c-fos or its protein product Fos in the medial prefrontal cortex (PFC). Administration of other antipsychotic drugs (APDs) does not induce Fos expression in the PFC. The mechanisms underlying the selective effect of clozapine on PFC expression is not well understood, but D₂-like, D₃-like, 5-HT₄, α₁, and muncarolin cholinergic receptors do not appear to be critical factors. We attempted to determine if lesions of key afferents to the PFC modify the cortical Fos response to clozapine. Lesions of the thalamic paraventricular nucleus, which projects to the deep layers of the infralimbic and prelimbic cortices, did not modify the clozapine-elicited increase in PFC Fos expression, as assessed by immunohistochemistry. However, lesions directed at the basolateral nucleus of the amygdala enhanced Fos expression in the PFC in response to clozapine challenge. The mechanisms subserving the modification of the cortical Fos response to clozapine are currently being examined. Supported in part by MH-45124, the VA Schizophrenia and Post-Traumatic Disorder Research Centers, and the National Parkinson Foundation Center of Excellence at Yale University.

The atypical antipsychotic clozapine (CLZ), the typical antipsychotic chlorpromazine (CPZ) and the putative antipsychotic olanzapine (OLANZAPINE (CLZ) were tested in rats trained to discriminate the serotonin (5-HT) antagonist metergoline (MIA, 4 mg/kg) from saline and in rats trained to discriminate the muscarinic antagonist atropine (SC, 0.25 mg/kg) from saline. CLZ produced full substitution in the MIA-trained rats with 87.5% drug-lever responding (DLR, 1.25 mg/kg dose) and partial substitution in the SC-trained rats with 60.5% DLR (5.0 mg/kg dose). The 2.0 mg/kg dose of CPZ produced partial substitution in the MIA-trained rats (58.5% DLR) but did not substitute for SC (18.0% DLR). CLZ produced a substitution profile intermediate between MIA and CPZ. The 1.25 mg/kg dose of OLANZAPINE produced 66.8% DLR in the MIA-trained rats and 36.0% DLR in the SC-trained rats. The selective 5-HT2C antagonist ritanserin produced complete substitution in the MIA-trained rats (98.4% DLR, 5.0 mg/kg dose) but did not substitute for SC (25.0% DLR, 2.5 mg/kg dose). Interestingly, there was an asymmetrical cross-generalization between MIA and SC. While MIA failed to produce SC--appropriate responding, SC produced 88.0% DLR in the MIA-trained rats. The present results demonstrated that the two-lever drug discrimination paradigm reveals different profiles for the atypical antipsychotic clozapine and the typical antipsychotic chlorpromazine in both MIA- and SC-trained rats. The putative atypical drug olanzapine produced a discriminative stimulus profile intermediate between that of CLZ and CPZ. Testing with other typical and putative antipsychotics will be necessary to establish the predictable validity of this behavioral screen for atypical antipsychotics.


and the force control variability of the haloperidol-induced movement may cause oculogyric crises in susceptible patients. We were interested in their influence on horizontal and vertical saccades in order to determine the qualitative or quantitative changes of such crises. To this end, a pilot study (open design) three healthy male volunteers (23-28 years old) received meticoo-pramid 10 mg tid. Eye movement registration with infrared oculography was done by application of the test substance, seven hours, and 22 hours after the first dose (six hours past the third dose). In two of the subjects the horizontal and vertical saccades were performed at the third measurement compared to baseline. In the second pilot study five healthy male volunteers received haloperidol 2 mg tid in a double-blind placebo-controlled design. The protocol of eye movement registration is described above. Four of the volunteers had slower saccadic peak velocities for horizontal and vertical saccades after the ingestion of haloperidol compared to placebo. D2-antagonists probably decrease the peak velocity of saccades unspecifically through their sedating effects. Regarding the oculogyric crises we conclude that there is no specific action of D2-antagonists on vertical eye movements and that the tendency of some patients to develop oculogyric crises on these drugs is due to differences in density and proportion of receptor-subtypes.
LOW-DOSE SLOWING OF RATS' LAPPING RHYTHM AS A POTENTIAL MARKER FOR ATYPICAL NEUROLEPTICS.

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The acute behavioral effects of the atypical neuroleptic clozapine (0.5-4.0 mg/kg), were compared with those of olanzapine (0.25-2.0 mg/kg) and risperidone (0.04-0.08 mg/kg). As thrity rats (n=29) lapped water from a force-sensing dish, the force of tongue contact was continuously recorded and then Fourier analyzed to yield estimates of the lick rhythm (in Hertz). All three drugs significantly, and dose-dependently reduced the lick rhythm, and the lowest dose of each drug had this effect even when the total number of licks per 2-min session (the lick index) was not significantly reduced. Neither the typical neuroleptic haloperidol nor the selective D2 dopamine receptor antagonist raclopride produced this slowing effect. The doses producing half the maximal effect on rhythm slowing for risperidone, olanzapine, and clozapine were 0.06, 0.95, and 1.16 mg/kg, respectively. The rank order of potencies suggested a serotonin-2 receptor involvement in the slowing of lick rhythm. Supported by MH43429.

DOPAMINE D3 RECEPTORS MEDIATE CLOZAPINE-INDUCED C-FOS EXPRESSION IN THE FOREBRAIN.

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The atypical antipsychotic clozapine produces a unique pattern of c-fos expression in the forebrain. While the receptor mechanisms by which this occurs are not well understood, there is some evidence to suggest that D2-like receptors may be involved. To determine if D3 receptors contribute to clozapine's effects on regional c-fos expression, in situ hybridization histochemistry with a cRNA oligonucleotide probe was used to identify cells that synthesize D3 receptor mRNA. Consistent with previous studies, D3 receptor mRNA was enriched in the islands (IC). In the IC, a moderate density of D3 mRNA signal was observed in the lateral septum (LS). In immunostaining with the probe in situ hybridization histochemistry demonstrated that most clozapine-induced fos- positive neurones in theicum were localized to the LC and D18, a moderate density of D3 mRNA signal was observed in the lateral septum (LS). In immunostaining with the probe in situ hybridization histochemistry demonstrated that most clozapine-induced fos- positive neurones in the IC and nucleus accumbens (NAc) contain D3 receptor mRNA. Further studies with oligonucleotide probes for endothelin and dystrophin showed that these regions do not contain D3 mRNA signal. The majority of these neurones in the LS, and nucleus accumbens (NAc) contain D3 receptor mRNA. In the IC, many fos-positive neurones express dystrophin while very few express endothelin RNA. A previous study has demonstrated that clozapine-induced c-fos expression in the IC, LS and NAc is blocked by D3 receptor agonists, suggesting that clozapine's effects in these regions are due to its antagonistic action at D3 receptors (Neurosci. 65, 3, 747). The co-localization of D3 receptor mRNA and Fos protein following clozapine administration in limbic brain is consistent with this hypothesis.

CLOZAPINE (CLOZ) PLUS SKF38939 STIMULATES THE ACTIVITY OF DOPAMINE (DA) DEPLETED RATS.

D. M. Jackson, N. Micheli, A. Bregni-A.

The aim of the present experiments was to investigate the locomotor-stimulant effects of the atypical antipsychotic agent CLOZ in rats depleted of their DA by reserpine and α-methyl-p-tyrosine pretreatment. CLOZ itself induced a slight but non-significant change which was enhanced by the selective D1 agonist SKF38939 but not by the selective D2 agonist quinpirole. The activation produced by CLOZ plus SKF38939 was partially blocked by the D2 antagonist SCH23390 but not by the D1 antagonist SCH23390, ineffective. A combination of SCH23390 and haloperidol completely blocked the locomotion effects of CLOZ plus SKF38939 induced locomotion. Unlike the effect seen with CLOZ, neither the 5-HT4 antagonist SCH23390 nor the D2 antagonist SCH23390 alone or in combination with SKF-38939. The locomotion effects of CLOZ were not altered by the combination of haloperidol and SCH23390. The data indicate that CLOZ, in rats depleted of their DA stores, exhibits properties similar to those of a D1 agonist. The pharmacological profile of CLOZ, however, is distinct, in particular, to that seen with the muscarinic antagonist scopolamine. The DA agonist like behavioural effects of CLOZ reported here may therefore be due in part to a muscarinic receptor antagonist like effect.


BW121055/R0, (+)-cis-2-(4-(2,1-benzisoxazol-3-yl)-1-piperazinyl)butyryl-4-A, 8,6,7,8,6-hexahydro-1H-pyrrolo[2,3-b]pyridine hydrogen sulfate, has been identified as a potential atypical antipsychotic drug (AP) (Rigdon and Norman, Neurosci. Abs. 20:1659, 1994). It binds to 5-HT4 sites with greater affinity than to D2, and has acceptable efficacy for the 5-HT4, D2, and α1-NIE. This study assessed the behavioral effects of BW12105 alone and with apomorphine (Apo) in a non-human primate screening model for AP. Four females from a colony of 5 stumptail macaques (Macaca nemestrina) were assigned to 2 treatment groups of 2 females each. After determining baseline behavior, each group received drug treatment for 2 days/week in a cross-over design. First, Apo was given alone, 1 mg/kg, i.m. at 1045-1100 hours daily for 2 days. Then, each group received BW12105 doses of 0.1-0.3 mg/kg, n.g. were given at 0800 and 1800 on Day 1 and on Day 2. Two 1 hr observation sessions were conducted at 0800 and 1100 each day to observe the effects of BW12105 alone and with Apo respectively. On Day 2 of treatment BW12105 produced a dose-dependent antagonism of the Apo-induced increase in locomotion levels produced by treated monkeys and checking (visual scanning). However, BW12105 failed to robustly antagonize Apo-induced stereotypes and produced only low levels of movement abnormalities at doses ≥1 mg/kg when given alone. Antagonism of increased submissiveness and checking is similar to known AP. The more than 10-fold separation between antagonist dose and movement disturbance threshold dose and weak antagonism of stereotypes is comparable to clozapine. Thus, the behavioral profile of BW12105 in primates is consistent with that of an atypical AP. (Supported by a gift from Burroughs-Wellcome, Research Triangle Park, NC).

MUSCARINIC m4 RECEPTOR AGONISM BY ATYPICAL BUT NOT TYPICAL ANTIPSYCHOTICS. X.P. Zeng, F. Le, L. Scarrabrick, and E. Richardson. Neuropharmacology Laboratory, Mayo Clinic Jacksonville, Florida 32224.

The muscarinic m4 receptor agonist effects of clozapine as recently reported by Zorn et al., 1994, may be related to its therapeutic and side effects as an antipsychotic drug (Tandon & Kane, 1993). We have further examined the effect of clozapine on forskolin-stimulated cyclic AMP accumulation in Chinese hamster ovary cells (CHO) expressing human m4 receptors to determine the possible relationship between m4 agonism and their pharmacological efficacy. Comparable to carbachol, at 1 μM, all the antipsychotics tested - clozapine, olanzapine, fluphenazine, clozapine, fluphenazine, and thiothixene - caused a 20 to 50% inhibition of forskolin (500 μM)-stimulated increase of cAMP. These effects were totally reversed by pretreatment with 10 μM atropine. In contrast, a series of typical neuroleptics - chlorpromazine, fluphenazine, molindone, and thiothixene - did not show the inhibition. In addition, none of the drugs tested caused a significant change in the basal concentration of cAMP. Therefore, m4 receptor agonism appears to be a common mechanism for atypical antipsychotic drugs, which may explain differences in the therapeutic efficacy and side effects between atypical and typical drugs. (Supported by Mayo Fdn. and USPHS GM37692)


ANTAGONISM OF PCP-INDUCED DEFICITS IN PREPULSE INHIBITION BY OLANZAPINE. V.P. Bañuelos and M.A. Gevirtz. Dept of Neuroscience, UCSD, La Jolla, CA 92037.

Presentation of a weak stimulus (the prepulse) immediately prior to an intense startling stimulus (the pulse) results in an attenuation of the startle response to the pulse. This phenomenon of prepulse inhibition (PPI) is thought to be a measure of sensorimotor gating mechanisms, which are deficient in schizophrenia patients. PPI is disrupted in schizophrenia patients and in rats, PPI is disrupted by the psychotogenic noncompetitive NMDA antagonist MK-801. However, the addition of the selective D1 agonist SKF38939 but not by the selective D2 agonist quinpirole. The activation produced by CLOZ plus SKF38939 was partially blocked by the D2 antagonist SCH23390 but not by the D1 antagonist SCH23390, ineffective. A combination of SCH23390 and haloperidol completely blocked the locomotion effects of CLOZ plus SKF38939 induced locomotion. Unlike the effect seen with CLOZ, neither the 5-HT4 antagonist SCH23390 nor the D2 antagonist SCH23390 alone or in combination with SKF-38939. The locomotion effects of CLOZ were not altered by the combination of haloperidol and SCH23390. The data indicate that CLOZ, in rats depleted of their DA stores, exhibits properties similar to those of a D1 agonist. The pharmacological profile of CLOZ, however, is distinct, in particular, to that seen with the muscarinic antagonist scopolamine. The DA agonist like behavioural effects of CLOZ reported here may therefore be due in part to a muscarinic receptor antagonist like effect.
667.17

INTRA-ACCUMBENS CLOzapINE AND HALOPERIDOL ENHANCE LATENT INHIBITION IN RATS. L.A. Dunn* and R.J. Schilb. Department of Psychiatry, Duke University Medical Center, Durham, North Carolina 27710.

Latent inhibition (LI) is an indirect behavioral measure of selective attention that is sensitive to dopaminergic and serotonergic receptor modulation. Haloperidol enhances LI across a broad dose range. Clozapine enhances LI only at very low doses (0.1 and 0.3 mg/kg) and reduces LI at moderate (0.3 mg/kg) doses (Dunn and Schilb, submitted). The anatomic locus of these effects is being explored. Sprague-Dawley rats weighing 150-175 gms were fitted with bilateral chronically implanted cannulae just anterior to either the nucleus accumbens (coordinates from bregma: A=2.2 mm, L=1.5 mm, V=7.2 mm, head 5° above intraluminal) or the amygdala (coordinates from bregma: P=0.3 mm, L=1.4 mm, V=8.1 mm, head 5° above intraluminal). Six days following surgery rats were tested in a LI measurement procedure published previously (Dunn et al., 1993, Psychopharmacology, 112:315-323). This consisted of 4 days of lick training, 1 day of preexposure (20 or 0 light stimuli) and conditioning (2 light, shock pairings), and 1 day of testing. Haloperidol, 50 ng in 1 μl, or clozapine 1 μg in 1 μl was administered once 2 minutes prior to preexposure. Vehicle was 0.3% w/v tartaric acid pH adjusted to 6.8. Haloperidol enhanced LI in both the nucleus accumbens (p<0.02) and the amygdala (p<0.05). Clozapine enhanced LI in the nucleus accumbens (p<0.05), but reduced LI in the amygdala (p<0.05). The divergent effects of clozapine in these nuclei is likely due to a differential sensitivity to 5-HT2A/c antagonism. (Supported by NIMH 47920)

667.18

CONTRASTING EFFECTS OF CHRONIC CLOZAPINE, SEROQUEL AND HALOPERIDOL ADMINISTRATION ON ΔF/ΣO EXPRESSION IN THE FOREBRAIN. F. Amaro* C. Y. Nakagome and G.S. Robertson, Dept. of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5. Dept. of Biochemistry, Medical Institute of Bioregulation, Kyushu University 812, Fukuoka 812.

We have recently demonstrated that specific neuronalanatomical patterns of increased Fos-like immunoactivity (FLI) are predictive of atypical antipsychotic-induced hyperkinetism. However, this effect has been administered chronically in order to generate both extrapyramidal side effects (EPS) and an optimal therapeutic response calls into question the relevance of acute changes in FLI for these long-term developments. In resolution of this problem, we have discovered that the immediate-early gene product ΔFosB is expressed in neurons activated by chronic alterations in dopaminergic neurotransmission. Chronic administration of typical and atypical neuroleptics may therefore produce distinct patterns of ΔFosB expression that can be used to identify such compounds and their neuronal targets.

To test this hypothesis, we compared the effects of chronic haloperidol, clozapine and seroquel administration on ΔFosB-like immunoactivity (ΔFosB-LI) in the rodent forebrain. Administration of haloperidol (2 mg/kg/day) for 19 days dramatically elevated ΔFosB-LI in both the dorsolateral and ventral striatum. In contrast, administration of either clozapine (20 mg/kg/day) or seroquel (10, 20 mg/kg) for 19 days elevated ΔFosB-LI primarily in limbic regions such as the prefrontal cortex, ventral striatum and lateral septal nucleus. These patterns of chronic increased ΔFosB-LI are similar to those observed acutely for FLI suggesting that ΔFosB may be a relevant marker for neuronal populations activated by prolonged neuroleptic administration.

AGING: PRIMATES INCLUDING HUMANS

668.1

VARIES THE TRACE INTERVAL IN EYEBLINK CONDITIONING IN YOUNG AND AGING HUMANS. M.C. Caporale, C.T. Fergusson & L. Dunnett, CM. Biology, University of Toronto, 1205 Health Sciences Mall, Toronto, Ontario, Canada.

Eyeblink conditioning has been used extensively to investigate the neural substrates of associative learning. Recent studies have found the effects of age and intramellar interval in delay conditioning to change over life span (Solomon et al., 1991). Other studies of trace conditioning have found nonoptimal intramellar intervals (Woods et al., 1991). Our goal was to determine the optimal trace interval in the eyetrackle conditioning paradigm for young and aging subjects, and subsequently test medial temporal lobe amnesia and Korsakoff amnnesia with the optimal interval.

Younger (n=32, 20-38 yrs) and older (n=32, 67-75 yrs) humans were randomly assigned to one of four trace intervals: 250, 500, 750, and 1000 ms. Eyeblink conditioning was given in an interval of 85 db, 100 ms tone following a 200 ms trace interval by a 100 ms, 3-pi corneal air puff sufficient to elicit reliable unconditioned responses. Sessions consisted of 30 preexposure trials (impaired tone and puff presentation), 60 conditioning trials, and 30 tone-alone extinction trials. A correction method was used to eliminate responses that could contamination data, including voluntary or alpha responses and random titling.

No aging difference in mean percent conditioned responses was observed for the 250 ms ISI, Young vs.52.6%, Aging=37.6%. Significant aging differences were found for the remaining three intervals: Young=55.8%, Aging=40.3%, 750 Young=34.6%, Aging=19.6%, Young=21.8%, Aging=10.7 (p<0.05). These data suggest that the trace eyeblink conditioning interval which resulted in the highest conditioning performance for young and aging humans was 500 ms. Having an optimal trace interval for young, as well as aging subjects is relevant when testing patient populations which vary in age. Currently, medial temporal lobe and Korsakoff amnesia are being used using the 500 ms trace eyeblink conditioning paradigm. Supported by AG 08796 to JFD, GM 17223-01 to MCC, RR-87048 to CRC NMH.

668.3

MRI DETECTED CEREBELLAR ATROPHY DURING AGING. Michael P. Sullivan, Leidy de Toledo-Morrell, Frank Morrell, and Sandra Spencer. Departments of Neurological Sciences, Psychology and Diagnostic Radiology, Rush Medical College, Chicago, IL 60612.

Classical, delay conditioning of the eyelid response has been shown to be impaired in aged individuals, thus implicating cerebellar dysfunction. With the use of high resolution, quantitative, magnetic resonance images (MRI) we hypothesized that a reduced volume of the cerebellum would be detectable when quantified in vivo, the extent of age or disease induced alterations in given brain regions of interest. In the present study, the presence of cerebellar atrophy was assessed using MRI. Healthy aged individuals were part of a larger study designed to examine the effects of aging on brain anatomy in vivo. 37 aged (mean age=70, range=61-84) and 27 young (mean age=27, range=23-35) were studied. The high resolution T1 MRI protocol. An interactive, 3-D reconstruction program was used to calculate the volume of the cerebellar regions of interest. Cerebellar volume was derived from 1885, 6mm sagittal slices, 9 on each side of the midline. To correct for individual differences in brain size, each person's absolute cerebellar volume was divided by the total intracranial volume, which was computed from sagittal slices spanning the whole brain. Cerebellar volume was found to be significantly reduced in aged subjects compared to young ones only when either absolute or normalized values were considered (t=3.414, df=62, p<0.001 for absolute cerebellar volume and t=4.044, df=62, p<0.001 for normalized volume). There were no gender differences in the amount of cerebellar reduction. Since cerebellar integrity is crucial for eyelid conditioning, this age-dependent volume loss may explain the behavioral deficits.

Supported by NIA grants PO1 AG09466 and P30 AG10161.

668.4


The hippocampal formation (HF), which is critically involved in the acquisition of certain types of new information, is especially vulnerable to the aging process. Until recently, it has been difficult to directly document such vulnerability in humans and to resolve the question of whether or not HF changes are specific to the aging process. With the advent of high resolution, quantitative, magnetic resonance imaging (MRI) protocols, it has been possible to visualize detailed brain anatomy in vivo and to quantify age or disease induced alterations in human brains. As part of a larger investigation designed to examine the effects of aging on brain anatomy in vivo. 37 aged (mean age=70, range=61-84) and 27 young subjects (mean age=27, range=23-34) were studied. The high resolution T1 MRI protocol was used to determine the hippocampal volume from coronal slices taken perpendicular to the long axis of the hippocampus. Left and right hippocampal volumes were derived separately. Individual differences in brain size were corrected for by dividing each person's hippocampal volume by total intracranial volume computed from sagittal slices spanning the entire brain. The right HF, but not the left, was significantly smaller in aged males compared to their young counterparts (F=23.82, p<0.001 for the age x hemisphere interaction). These differences were greater in males than in females. Since cerebellar integrity is crucial for eyelid conditioning, this age-dependent volume loss may explain the behavioral deficits. (Supported by NIA grants PO1 AG09466 and P30 AG10161.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
668.6 Aging-related deficit in implicit perceptuo-motor learning. E. C. McNay* & D. B. Willingham, Neuroscience Program, University of Virginia, Charlottesville, VA 22903.

A widely-held view of the basis of age-related deficits in learning and memory has been that deficits are caused by a reduction in the level of resources available for working memory, self-initiated thinking, and the like, that is part of some other model. This has been offered as an explanation for the observed reduction in performance with aging on explicit tasks and spared performance on implicit tasks.

We investigated the effect on aging on both explicit and implicit measures of perceptuo-motor learning. Subjects were tested using a task which required them to trace a line presented on a computer screen using a pen held out of their sight. Baseline measurements were taken, followed by two experimental phases. In the training phase, the feedback on the screen of the subjects' movements was transformed (a constant 90 degree rotation). Subjects were told that there was a transformation, but not of the identity of the transformation. After each block of training trials, both transformation and feedback were removed for a block of test trials in which subjects were asked to directly trace the line presented. The primary measures of performance were deviation from target line (training phase, explicit measure) and signed deviation from target line (test phase, implicit measure of space recalibration). Contrary to expectations, aged subjects showed impaired performance (after correction for age-related changes in drawing ability) in both phases, with greater deviation from the target line in the training phase and lower recalibration of their perceptuo-motor space in the test phase.

668.7 SPATIAL MEMORY AS MEASURED BY A HUMAN MAZE IN AGED SUBJECTS SHOWING VARIOUS PATTERNS OF CORTISOL SECRETION AND MEMORY FUNCTION.

L. Sunier, T. Nip, C. Ramnial, V.P. Nair, R.J. Hauger, M.J. Mealey
Research Division, St. Joseph's Healthcare, Queen Mary, Mississauga, ON, Canada, H5W 1S3; Douglas Hospital Research Centre, McGill University, Montreal, Canada, 685 Blvd. Lionel, Verdun, Quebec, H4H 1R3.

Studies have shown that hyposecretion of corticosteroids result in hippocampal spatial memory deficit and spatial memory dysfunction in the aged rat. We have recently shown in a group of elderly human subjects that a significant increase of cortisol levels over a period of 3 to 6 years present an amnestic profile as revealed by a poor declarative memory performance while aged subjects shows a moderate increase or a decrease in cortisol levels with years perform normally on these tasks. We have tested spatial memory performance in this aged population using a human maze. Three types of encoding were used for the memory measures: a spatial encoding for which the subject was shown a path by following the experimenter through the maze and was then required to do it on his own; a verbal encoding for which the subject was given verbal instructions to follow in order to find his way in the maze; a contextual encoding for which the subject had to create a cognitive map of the maze in order to perform the task. Correlational analyses were performed between the cortisol slope of subjects as measured over a period of 3 to 6 years and their performance on the three types of encoding. The results showed significant positive correlations between the cortisol levels and the time the subjects spent in the spatial (r=0.75) and verbal encoding (r=0.45) conditions, with no significant correlation between the cortisol slopes and the contextual encoding condition (r=0.08). Since this last encoding condition did not involve any memory processing while the spatial and verbal encoding did, these results suggest that the amnestic profile described in aged subjects showing a significant increase of cortisol levels with years applies to both spatial and non-spatial information. This further confirm the animal data literature which reports a significant relationship between prolonged exposure to corticosteroids in later life and memory dysfunction.

668.8 A LONGITUDINAL STUDY OF DEHYDROEPİandrosterone SULFATE (DHEA-S) LEVELS, CORTISOL LEVELS AND COGNITIVE FUNCTION IN ELDERLY HUMAN SUBJECTS.

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It has been postulated that dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are markers of successful aging and life expectancy while cortisol levels may be markers of stress. We have previously shown in a longitudinal study of elderly volunteers that the slope of the increase in cortisol secretion over years predicts the nature and the magnitude of the cognitive decline. We have retrospectively analyzed DHEA-S levels in this population of aged individuals for the years 1990-91, 1991-92 and 1992-93 in order to determine if DHEA-S secretion is negatively correlated with cortisol hypersecretion and cognitive deficits. Subjects were resident in year 1992-93 for 24-hour cortisol secretion as well as memory function and we replicated the cortisol/memory impairment relationship previously reported. The results of the analysis of DHEA-S levels revealed decreasing DHEA-S levels in all subjects. Consistent with the hypothesis that DHEA-S may have a protective effect on the aging system, we have shown that subjects showing a significant increase in cortisol levels with years and memory impairment had the greatest decrease in DHEA-S levels. However, the reverse pattern was not observed in individuals showing decreasing cortisol levels and highly efficient cognitive functions. These individuals consistently presented lower DHEA-S levels than the other subjects. Finally, although cortisol levels were significantly correlated with cognitive function, DHEA-S levels were not significantly correlated with cognitive efficiency. These results suggest that cortisol secretion during aging may be a better biological marker of cognitive decline than DHEA-S secretion.

668.9 DEMENTIA AND RESPONSE TO PAIN IN THE ELDERLY. F. Porter, M. Smith, J.P. Miller, J. Morris*
Washington University School of Medicine, St Louis, MO 63110.

To study relations among dementia, pain response and perception, heart rate and facial expression were continuously recorded during undisturbed BASEline (13x3mm), PREparation (turquoise/cleansing needle visualization) (1 mm), VENipuncture (2x3 mm), and undisturbed RECovery (10x3mm) from 44 (24 female) adults (mean age=73±9 years) during a standard unanesthetized venipuncture. Clinical dementia ratings (0=Normal, 3=severe dementia) were assessed just before, during and after VENipuncture, and the subject was assessed with H.R. and D.M. DHEA was increased from BASE to PREP and decreased from PREP to VENI (p<0.001), returning to BASE levels during VENI. A lower magnitude H.R. response to PREP and during VENI was associated with dementia but not with age or gender. Higher general pain sensitivity and higher anticipatory anxiety were positively correlated with the magnitude of H.R. response to VENI and negatively correlated with D.M. sensitivity, but <40% of demented subjects were able to provide ratings. Demented subjects exhibited more facial movements to PREP (p<0.05) and VENI (p<0.02) than normals. More facial movements during BASE were associated with less H.R. increase to PREP. We conclude that the elderly exhibit a larger H.R. increase to stress (PREP) than pain (VENI) and that dementia may: 1) decrease H.R. response to stress and pain, 2) minimize the magnitude of D.M. response to stress and pain, and 3) increase facial expression to pain. Thus, there is an apparent discordance among behavioral, physiologic and self-reported reactions to stress and pain among the demented. (NIA PS0 AG05868).


Healthy elderly experience "pop-out" in visual search as readily as do young subjects when the target is easily discriminable from distractors. However, search for a poorly discriminable target, believed to require the movement of visuospatial attention from one display element to the other, proceeds more slowly in the healthy elderly compared to young subjects (Flude & Dousnass-Roosevelt, 1989; Greenwood et al. 1992). Does this arise from a different spatial distribution of visuospatial attention in aging? Young subjects and two groups of older adults (aged 65-74 and 75-80) were trained to exclude memory loss and dementia searched a display of 15 letters for a target defined by a conjunction of color and letter (a pink "K") and surrounded by 5.57 or 9 target color (pinks) and non-target colors (green) in yellow green. In this type of search, subjects confine their attention to the target color items. Although RT slowed in all groups as the number of target color distractors increased, the increase was greatest in the elderly group. More eccentric target locations heightened the slowing of RT with increased numbers of target color distractors. This effect of eccentricity in slowing RT was greater in the elderly than in the young. However, when the target was centered in the display the young were unaffected by the number of target color distractors while the elderly were additionally slowed with each increase in target color distractors. This result suggests that the spatial distribution of visuospatial attention is more diffuse in the elderly than in the young. A broader focus of visuospatial attention may contribute to age-related (1) slowing in speed and (2) increased vulnerability of search to the presence of distractors.
SHIFTING ATTENTION BETWEEN SENSORY MODALITIES IN HEALTHY AGING. A. Berardi*, J.V. Harby, Lab of Neurosciences, National Institute of Mental Health, NIH, Bethesda, MD and Harvard, INI, London, UK.

Age-related changes in attention shifting were investigated by measuring reaction time (RT) to visual and auditory stimuli under two conditions: a visual vs. baseline task in which six subjects were tested at random using a stimulus presentation when five minutes to five minutes duration was used and 45 trials of 18 trials. Twenty-six subjects between the ages of 20 and 25, 31 subjects, and 60-64 years old and 60-64 years old, was 0 < 0.05 (for all comparisons between young subjects and older subjects). When reaction times on the shifting task were expressed as percent increase relative to the baseline task, the main group effects remained significant, indicating that age-related slowing on the shifting task was greater than would be predicted by generalized slowing. These results demonstrate that age-related deficits in shifting attention between sensory modalities exist in middle aged as well as in old subjects, and this is not attributable to generalized slowing.

THE EFFECTS OF ORIENTING TASK AND NUMBER OF TRIALS ON THE OLFACTORY EVENT-RELATED POTENTIAL IN YOUNG AND OLDER ADULTS. C.D. Morgan, J.W. Covington, C. Quiñonez, D. Ellison, D. Wester, D.L. Kalinski*, J. Polich, C. Murphy(UCSD Medical Center, Naval Medical Center, The Scripps Research Institute, San Diego State University, San Diego, CA 92120, Moorhouse Critical Senses Center, Philadelphia PA 19104)

Olfactory event-related potentials (ERP) are currently used to differentiate groups of individuals based on peak amplitude and latency and show considerable promise as a diagnostic tool in clinical settings. In an effort toward determining clinical utility in an individual participant, the present study was conducted with the goal of finding the optimal parameters for recording the most robust ERPs in both young and older adults. Using three different stimulus odors (amyl acetate, geraniol, phenylethyl alcohol) in the clearest and most controlled ERP waveform was measured and replicated by amyl acetate. The average of 20 stimulus trials recorded from each participant produced a robust waveform, however, a distinct wave was present in the average waveform for each subject and was found to be sensitive to shorter administration time, a factor which is desirable in clinical settings. Furthermore, peak amplitudes and peak latencies were found to be significantly different for both age groups despite that the amplitudes were small in older adults and latencies were longer for the older participants, indicating that age is a significant factor in OERP responses. These findings will help guide future research and clinical application in olfactory assessment.

Supported by NIH grant #SDC02064 to C. M.


We examined the size of the corpus callosum (CC), temporal cortex, parahippocampal gyrus, and hippocampus using quantitative MRI in 26 healthy older adults (mean age 87) and 10 elderly healthy old subjects (mean age 87). We examined the relationship between structure size and cognition. Of the 12 subjects showed cognitive decline (e.g., CDR = 5.1) in the subsequent four years of follow up (Incipient Dementia, ID). Therefore, we examined structure size in HE and ID subjects where the ID subjects were cognitively normal. The hippocampus was smaller in the ID subjects (p < .05). Temporal cortex correlated with CC size (R = .44, p = .03). The middle and posterior sectors of the CC were correlated with temporal cortex in the ID (R's > .55, p's < .05) but not the HE subjects. Significant relationships were found between memory and the hippocampal volume, and midsector of the CC over all subjects (R's > .45, p's < .03). These data suggest that atrophy precedes clinical signs of dementia. We are currently examining MRI and cognitive assessment data during subsequent years of cognitive decline in these subjects. [Supported by Dep't Veterans Affairs, NIH AG08017, NIH AG12611]
669.1
CORTICAL SYNAPTogenesis in HEMIgALENCEPHALY.
J.R. O'Rusky* and H.V. Vinters†. Dept. of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, B.C., Canada V5Z 1M9 and UCLA Medical Center, Los Angeles, CA 90024.

Historical and electron microscopic analyses of histological specimens of 3 cases of hemigaleenencephaly (HGE) and 4 control cases (Rasmussen's encephalitis) ranging in age from 1 to 7 years revealed that the number of asymmetric and symmetric synapses in the cerebral cortex were reduced (HGE) or normal (Rasmussen's). The number of asymmetric synapses decreased after 1 year of age due to a loss of axosomatic contacts with no change in the density of axodendritic or axosomatic contacts. The Ns of symmetric synapses increased gradually up to 7 years of age. In HGE cases the Ns of asymmetric and symmetric synapses did not differ significantly from controls. Given the increased cortical thickness in HGE, the number of synapses in a column of cerebral cortex beneath 1 mm of pial surface was substantially greater than in controls. (Supported by the B.C. Health Research Foundation)

669.2
SACCADIC VARIABLES IN ADULTS WITH CEREBRAL PALSY. M. LeGare*, S. Gu, B. Zhang and S. Lee. Biomedical Engineering Program, California State University, Sacramento, CA 95819-6019.

Aimed movements require visual localization. The slow and inefficient pursuit movements in cerebral palsy (CP) may be partly due to the saccadic system which provides visuomotor support for localization. Asymmetry of the left eye position of the 6 CP and 4 normal (N) adults were measured by the 70000 (Micromeasurements, Inc., Parmington, CT) at three frequencies (0.3-0.5-0.7 Hz), three amplitudes (14.16.18), horizontal and vertical dimensions were used for 18-19 tests in a J-saccadic paradigm. The right and left eye positions were pooled for this analysis of five saccadic variables. The CP group had greater than normal means for: saccadic latency (M-HGE=0.363±0.207; M-N=0.268±0.088), RMS error (M-HGE=3.568±1.69; M-N=2.680±0.88), and fixation (M-HGE=2.99±0.09; M-N=2.662±0.053). The CP group made more low velocity saccades than the N group but the acceleration profiles were comparable indicating similar organization. Therefore, the system should also exhibit motor learning. Such training may improve aimed movement in CP. Supported by: NSF-BCS9107726, CSUS-RA to ML.

669.3

We describe a unique case of complex craniofacial and CNS anomalies. The focus here is on the nervous system. The child's skull was asymmetric (plagiopcephalic) with the axis displaced to the right. However, her motor and distinguishing feature was that the left orbit contained two eyes while the right orbit contained one normal appearing and functioning eye. Accordingly the anomaly was termed TROPOCRANIA. Computer assisted medical imaging was used to create intra- and extraxial structures in vivo. There were two left cerebral hemispheres: the anterior was gyrencephalic; the posterior lissencephalic. The other abnormalities included: a right proptosis; the left lateral recessus on the left; two left ophthalmic arteries; two left middle cerebral arteries; agenesis of the corpus callosum; duplication of the fab cerebi, sagittal dural venous sinuses and confluens of sinuses. The circle of Willis was incomplete. The brain stem was nearly symmetrically with slight reduction in the left cerebellar hemisphere and an attenuated left medullary pyramid. We propose that the embryological basis for this complex anomaly was an early decrease in neuronal proliferation, in the neural plate, of primordia for the eye and secondary prosenecencephalic.

Supported by NIH Grant NS 17783, the McDonald Center for Studies of Higher Brain Function and an award from theSpecify Parkinson's Foundation of the Illinois-Eastern Iowa District of the Knock Francisco International

669.4
Marine Platelet Activating Factor Acetylhydrolase: Expression Pattern and Enzymatic Activity in the Developing Brain Correlate with the Timing of Neural Migration. U. Albrecht, R. Abu-Issa, L. Colohan*, K. Inoue and G. Eichele, Dept. of Biochemistry and Divs. of Neuroscience, Baylor College of Medicine, Houston TX 77030, USA. Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo Japan.

Miller-Dieker lissencephaly, a human brain malformation associated with a hemimegalencephalic deletion in the LIS I gene, is a defective cell migration leading to an abnormal cerebral cortex. Radio-immunoassay of marine platelet activating factor (PAF-AH) has been purified previously. PAF-AH deacylates and thereby inactivates platelet activating factor, an alkyl-ether phospholipid implicated in various aspects of neuronal development and in neural migration. Purification studies suggest that PAF-AH consists of three subunits (29kD, 30kD and 45kD) and the 45kD subunit is identical with the coding sequence of the LIS I gene. In order to study the spatial and temporal expression of the PAF-AH subunits, we cloned the cDNAs for the mouse 29kD and 30kD subunits. Both subunits are highly conserved between mouse, bovine and human. We find that the mRNA encoding the 29kD, 30kD and 45kD subunits are expressed in the cerebral cortex and in the hippocampus. Expression is also observed in the migrating granule cells of the developing cerebellum. PAF-AH activity measurements in cytosolic extracts show that in the cerebellum activity is highest between postnatal day 5 and 10, the time-span of granule cell migration. Correlation between elevated enzymatic activity and high levels of expression in the developing cerebellum substantiate the notion that 29kD, 30kD and 45kD subunits are associated in vivo.

669.5
SPECIFIC CORRECTION OF "ANTI-BRAIN" AUTOIMMUNITY. E.F. Bakunenko, B.B. Gnedenko, A.B. Polatov*. Chernobyl-Test Ctr., 10 Rimagko-Korakova St., 127377 Moscow, Russia.

As it was found earlier, most of children, which have been born from mothers with elevated serum anti-S100 immunoactivity, have revealed a different forms of the brain malfunctioning (mental retardations, cerebral palsy, seizure syndromes). Ability to abnormally production of anti-S100 antibodies (AB) has epigenetically transferred from mother (not father) to offspring and could be related to aggravation of the brain dysfunctions. It was found that maternal "tuning" of the offspring immune system on a combination of AB-dependent modulation of specific lymphocyte repertoire, and direct transfer, and persistence of maternal "memory" lymphocytes into fetus. In experiments (rats were preliminary immunized to S100) it was found that parenteral administration of antidiotype AB could inhibit specific serum anti-S100 reactivity. Also there were found two immunomodulatory S100 fragments. The first one had at least equal efficiency, as compared to their inclusion of specific T-suppressors?). The second one significantly increased anti-S100 immunity (probably due to stimulation of a specific T-helpers). These findings may explain some aspects of pathologic anti-S100 autoimmunity as well as to be used for elaboration of a prophylactic measures.

669.6
INDUCED MICROGYRIA AND ITS EFFECTS ON CELL SIZE, CELL NUMBER, AND CELLULAR DENSITY IN THE MEDIAL GENICULATE NUCLEUS. J.A. Herman, J. Hitch, J.A. Gagliardi*, and G.D. Rozin, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115 and CNBM, Rutgers Univ. Newark, NJ 07102.

Microgyria can be induced in otherwise normal rat neocortex by neonatal freezing injury (Humphreys et al., J. Neurophysiol. 59:4145, 1988). Adult animals with microgyria and somatosensory cortices show deficits in fast auditory temporal processing (Fitch et al., cereb. Cortex 3:240, 1993). It is not understood why damage to cortical areas far from auditory input would disrupt auditory discrimination. We have hypothesized that the developmental cortical injury results in disinhibition of the somatosensory related areas, and that some of these changes involve areas important for auditory processing.

To test this hypothesis we measured cross sectional cell areas, cell packing densities, and total cellular numbers in the medial geniculate nucleus (MGN) of rats who had received neonatal cortical freezing or sham injury and who had been behaviorally tested for auditory temporal processing in adulthood. Measurements of cross-sectional neocortical areas and cell packing density of ventral, dorsal, and medial regions of the MGN were derived using a variation of the point counting technique (Williams and Rakic, J. Comp. Neuphotol. 278:344,1988), and MGN volumes were computed based on estimates from serial sections.

Preliminary analysis has shown changes in the patterns of asymmetry in cell size between microgyric and control rats — control rats have larger neurons in the left hemispheric while microgyric animals show no asymmetry. Continued analysis will concentrate on gender differences, differences based on lesion location, and the relationship of the anatomic measures to auditory temporal processing.

This work was supported, in part, by grant HD19208.
669.7 NEOCORTICAL DYSPLASIA, MICROGYRIA, AND POTENCY OF COMMON ETOGENES? C.L. Rossen and P.L. Sherman, Dyslexia Research Laboratory, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115.

Injury to the developing cortical plate before the end of neuronal migration is generally believed to be the underlying cause of neocortical migration deficits, which result in microgyria and poteenophaly (Sarnat, A.M. Dis. Children 14:1-96, 1987). It has been hypothesized that different forms of developmental pathology result from distinct mechanisms of injury. Alternatively, they may result from a single mechanism, but only the severity of the injury varies among them.

Molecular layer ectopias and microgyria have been induced in otherwise normal mice by perfusing the developing cortex with cold saline or by placing a cell-penetrating probe on one side of the skull during the first day of gestation. These treatments result in final cortical layers 2 to 3 seconds. In some rats, the procedure was repeated a second time at a location adjacent to the first. The animals were sacrificed on either P2, P4, P10, P12, or P14 and brains were examined for immunohistochemical stains for the presence of neoposteival pathology. Single injury 5 seconds or longer resulted in the formation of microgyria, with the depth of the microcortex and the volume of the lesion increasing with longer freezing times. Two-second freezing injury rarely resulted in microgyria, and instead most often led to molecular layer ectopias and/or minor laminar dysplasia. As expected, injury at two adjacent sites led to more severe disturbances that single injury, culminating in the formation of poteenophaly cortex following 20 second injury. Those mice most affected can undergo the formation of molecular layer ectopias, microgyria, and poteenophaly.

Supported in part, by HD20806.

669.9 CONNECTIONAL VEXICITY OF ECTOPIAS IN AUTOMICINE MICE: A. R. Jenner, A. M. Gallaburda, and D. Sherman, Dyslexia Research Laboratory, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115.

New Zealand Black mice (NZB/N1) and NSX-M al mice develop minor neocortic malformations, characterized by the presence of ectopic neurons in the putamen of dyslexics. Approximately 40% of the NZB and 80% of the NSX-M mice prenatally develop clusters of ectopic neurons in cortical layer 1. Neuronal damage affecting these neurons provided preliminary evidence that the connections of these cells were absent. The present study was designed to look more specifically at the cortical topographic connections. Adult mice were perfused with 7% piaformaldehyde/1% glutaraldehyde and the brains removed from the skull. The brains were stained with methyl green in order to visualize the surface of the brain more clearly and viewed under the dissecting microscope for ectopia. Large ectopia appear as small bumps on the surface of the brain. These were visualized in seven brains. A small crystal of DiD which labels cell bodies was used as the dye of choice. In some cases, the midline was used in certain cases to visualize the DiD-labeled label in the cortex just under the pia surface. The brains were scored in two directions for up to 20 months. The brains were cut with a vibratome into 100 mm sections and viewed under a rhodamine filter set in order to visualize the DiD.

The specific connections of the DiD-labeled cells and in all cases there was a distinctive connection of labeled fibers extending from the ectopic cells through the layers of the cortex. This bundle of fibers then either entered the corpus callosus or the internal capsule. Six ectopia were visualized in the commissural barrel cortex and one was in the hindlimb sensorimotor cortex. Depending on the location of the ectopia within the bundle cortex labeling was seen in thalamic nuclei, and/or the hippocampus. This bundle of fibers was seen in the corpus callosus and visualized in the cortex commissural barrel cortex and second somatosensory and primary motor cortex. Two of these DiD studies provide the first conclusive evidence that the neurons within the cortex are connected both to other cortical afferent thalamic and thalamic nuclei. Supported by NIH grant HD 20806 & the Sackler Scholarship.


We have previously described the pathogenesis of massive pyramidal and granule cell loss in the hippocampi of mice transgenic for an amyloid precursor protein construct (APP-C100) driven by a neuron-specific promoter. When reporter sequence FLAG is added to the construct, we have observed extensive degeneration as early as one year of age in some of these transgenic lines. Lysosome-like inclusions, immunoreactive for the antibodies that recognize epitopes in the C-100 construct, appear in increasing numbers of cells and increasing numbers appear in individual neurons in the CA1 and CA3 areas by 1 year of age. Examination of cerebellum, cerebral cortices, spinal cord, hypothalamus, and striatum have revealed no significant neuronal cell loss relative to that seen in the APP-C100 mice. The degeneration observed is that of hippocampal formation of individual transgenic animals. In addition, many of the APP-C100 mice exhibit cardiac myopathy, hepatosplenomegaly, and extramedullary hematopoiesis isochromatid rearrangements occurring in human beings with anaplastic leukemia or in individuals with Down Syndrome.

The earlier and more rapid degeneration of the hippocampus in the FLAG construct is more frequently by megayacrophagy abnormalities which may be associated with the platelet abnormalities observed in individuals with DS and some individuals with AD. Supported by HD19832 (MLOG) and NS28688 (RN).
669.13

ALZHEIMER'S TYPE NEUROFIBRILLARY DEGENERATION IN BRAIN WARTS M. A. Moran* and P. Gomez-Ramos Morphology Dep., Autonomous Univ. Sch. of Med., Madrid, Spain

Verroustiasias (VD) of the cerebral cortex are developmental abnormalities characterized by a nodular shape and a disrupted cytoarchitecture of upper layers. In three autopsied elderly individuals (two without neurologic disorders and one with motor neuron disease), VD presented abundant Alzheimer's type neurofibrillary degeneration (ND), which immunoreacted with 5E2 and AT8 Abs, disclosed cholinesterase activity, and were thioflavin-S positive. This ND was conspicuous in layers II and III abutting the external borders of the dysplasia, as well as in layers V, VI, and in the underlying white matter. All VD contained cell-sparse areas within them, around which a peak of ND was seen. We suggest that alterations in the neuropil around neurons disturbed in their migration make them vulnerable to undergo cytoskeletal changes. Additional micro-environmental anomalies related to hypoxia-ischemia in these lesions are proposed as contributing factors for ND, pointing to the potential of VD as a source of material to study unresolved issues such as the way ND may be related to ischemia. Supported by F.I.S. 93/0198 Spain.

669.15

ALTERED MEMBRANE RESISTANCE AND REDUCED OUTWARD CONDUCTANCE IN CULTURED TRISOM 16 MOUSE TONGUE MUSCLE CELLS SUPPORT A MECHANISM FOR THE HYPOXIA IN DOWN SYNDROME S. Peng, Z. Goldschick*, and S. I. Rapoport Lab of Neuroenceology, NIA, NIH, Bethesda, MD 20892

Trisomy 16 (T16) mouse is a genetic animal model of Down syndrome (DS; human T21). Young DS patients develop a characteristic hypoxia. It has been reported that the tongue muscle is abnormal in DS. A whole-cell patch-clamp method was used to study membrane properties of isolated T16 and diploid control tongue muscle cells. Muscle cell cultures were prepared from diploid and T16 mouse fetuses at the 10th day of gestation. Cells were maintained for 21 or 23 days. Electrophysiological recordings were done in vitro in normal physiological solutions with 1 mM OCL, on cells cultured between day 7 and 14. Before recording, cells became spherical after a few hours of exposure to 0.2 M colcemid. Membrane resistance (Rm) and capacitance (Cm) were measured at a holding potential of -60 mV. Mean T16 cell Rm was 372 MΩ (±15; n=17), whereas Rm of control was 513 MΩ (±39; n=31). Thus the T16 cell Rm was 27.5% less than that of control. We did not detect significant differences in Cm between T16 and control cells. Furthermore, we applied 15 steps of depolarization voltage with 10 mV increments to elicit total membrane current at holding potential of -60 mV. The mean of normalized maximum outward conductance (kmax/Cm) was 497 pS/pF (±60; n=17) and 843 pS/pF (±81; n=31) for T16 and control cells respectively. Thus the kmax/Cm of the T16 cells was 41% lower than that of control, but there was no significant difference in inward current conductance. Therefore, the cause of hypoxia could be the increased Cm conductance.

BETA-AMYLLOID: ApoE II

670.1

RATS TREATED WITH ANTISENSE OLIGONUCLEOTIDES TO APP AND APoE RETAIN PLACE, CUE, AND MOTOR MEMORY. C.A. Montez*, S. Argawal*, D.P. Bisack* Dept. of Psychiatry and Human Behavior and Dept. Neuroscience Brown University, Providence RI, 02912; and, Hybond, Inc., 172, Worchester, MA 01605

Antisense compounds can be applied to modulate the expression of gene products implicated in a variety of disorders, including Alzheimer’s Disease (AD). We designed phosphorothioate oligonucleotides complementary to amyloid precursor protein (APP) mRNA and to apolipoprotein E (APO-E) mRNA. In a previous report we showed that anti-APP can modulate the expression of APP in PCH12 cells (Cell: Molec. Neurobiol. 14:425-437; 1994). The most common symptom of AD involves the disruption of memory. Thus, any potential therapeutic agent requires initial assessment with respect to its impact on behavioral functions normal males. Male rats were injected (i.v.) with: 1) anti-APP; 2) anti-APO-E; 3) saline; or 4) unrelated (sense) oligonucleotides at dosages of 2.5 or 5.0 mg/kg. All rats were trained in a multi-component water maze task which simultaneously assessed long-term and short-term place memory, cue memory, and motor memory. Antisense oligonucleotides produced no deleterious effects on normal rats when tested for place, cue, and motor memory at the dosages tested. Safety and efficacy assessments are continuing to evaluate the therapeutic potential of antisense compounds in the treatment of AD.

670.2

EXPRESSION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR FAMILY MEMBER gp300 IN RESPONSE TO EXPERIMENTAL LESION MODELS OF ALZHEIMER DISEASE: G.M. Fasano1*, M.Z. Kowman1, W.S. Argawal2, and C.E. Finch3, 1Division of Neurogerontology, Andrus Gerontology Center and Dept. of Biological Sciences, USC, Los Angeles, CA 90089, 2J.H. Holland Lab, Dept. of Biochem., American Red Cross, Rockville, MD 20855, 3Alzheimer’s Disease Research Center at University of California, Los Angeles, CA 90024

Alzheimer’s disease contains the apolipoproteins apoA (clustering) and apoE in complexes with the amyloid β-peptide (Aβ), which could have roles in the metabolism of Aβ. The LDL-receptor family contains a shared binding site for both apoA and apoE (Kounnas et al., J. Biol. Chem. 1995, in press), whereas the LDL receptor related protein (LRP) binds apoA but not apoE. ApoELRP has been linked to AD-related neuritic plaques and brain lesions that model features of Alzheimer disease. During responses to hippocampal deafferentation by perforant pathway transection, gp300 mRNA was induced in hippocampal neurons of the CA1-C4 subdivision and subiculum. Similarly, after infusion of synthetic aggregated Aβ(1-42) peptide, surviving hippocampal neurons showed elevated immunoactivity for the class II MHC antigen. The findings highlight the coexpression of gp330 and one of its ligands apoA at sites of brain injury which is consistent with the possibility that gp330 may be functioning to modulate clearance of apoA at these sites. This work was supported by the National W. & Margaret T. Shock Aging Research Foundation to GMP by the NIA (AG-07909) to CEF and NIH (DK55586) to WSA.
APP751 (PROTEASE NIN2): PROTEASE COMPLEXES ARE INTERNALIZED VIA THE LDL RECEPTOR-RELATED PROTEIN (LRP)

**BINDING OF $\beta$-AMYLOID PEPTIDE (AP) TO APOLIPOPROTEINS E3 AND E4:** J. M. Shaffer*, N. J. Reiner-Cook, R. Gupta-Banali and K. E. Brandt, Glialtech, Inc., Cleveland, OH 44122.

Apolipoprotein E (apoE) is found associated with senile plaques in Alzheimer's disease (AD), and recently report data reveal that individuals expressing the apoE isoform have an increased probability of developing AD. Previous studies demonstrated that apoE4 and the more common apoE3 isoform both bind AB, the major component of senile plaques. However, only SBS and heat stable complexes were quantified, and conflicting results were obtained regarding the relative amounts of these complexes. Since there is a likelihood that $\beta$-amyloid might form SBS-labile complexes with AB, we have utilized a solid-phase binding assay to determine the affinity of recombinant apoE3 and apoE4 for fibrillar AB. The apoE isoforms were immobilized in 96-well plates, and fibrillar preparations of AB were subsequently allowed to interact with the coated wells. Bound amyloid peptide was detected with a specific monoclonal antibody, followed by the addition of a peroxidase-conjugated anti-mouse antibody. AB showed saturable binding to both apoE3 and apoE4. There were not dramatic differences in the affinity of AB for either apoE isoform at pH 7.4, with Kd values of approximately 100-200 nM. To ensure that immobilization of apoE did not affect antibody binding sites, experiments were performed in which increasing concentrations of apoE3 and apoE4 were added to the binding solutions to compete for AB associated with immobilized apoE. A dose-dependent inhibition of AB binding was seen with solution-phase apoE3 and apoE4, with Kd values that were in general agreement with the Kd values mentioned above. These data suggest that both apoE3 and apoE4 may be involved in the fibril formation. As a result, further work is needed to determine the role of each apoE isoform in the deposition of AB.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of amyloidosis. The main component of amyloid deposits is the 42 amino acid peptide referred to as "Aβ". AD is associated with an increased frequency of the apolipoprotein E (Aplo) e4 allele. Apolipoprotein E is a secreted glycoprotein involved in cholesterol transport and systemic outgrowth. AD, ApoE is also found in amyloid deposits. Apolipoprotein E binding to Aβ could lead to their aggregation into amyloid fibrils. Conversely, Apolipoprotein E allele may be protective against AD by inhibiting amyloid deposition in brains of AD patients at different stages of amyloid deposition including early and late dementia and 40-semester cases. Apolipoprotein E allele may be protected against AD by inhibiting amyloid formation and fibrillogenesis in AD patients. The results of this study show that ApoE is a good biomarker for Alzheimer's disease. ApoE is also a good therapeutic candidate for the treatment of AD. ApoE may be a good target for the development of new therapies for AD.

PHYSIOLOGICAL AND BIOTECHNICAL QUANTIFICATION OF APOE.”

BETA-AMYLOID: "B" by mechanically stable peptide outgrowth. The presence of ApoE in AD increases the risk of Alzheimer's disease. The presence of ApoE in AD is associated with an increased frequency of the apolipoprotein E (Aplo) e4 allele. Apolipoprotein E is a secreted glycoprotein involved in cholesterol transport and systemic outgrowth. AD, ApoE is also found in amyloid deposits. Apolipoprotein E binding to Aβ could lead to their aggregation into amyloid fibrils. Conversely, Apolipoprotein E allele may be protective against AD by inhibiting amyloid deposition in brains of AD patients at different stages of amyloid deposition including early and late dementia and 40-semester cases. Apolipoprotein E allele may be protected against AD by inhibiting amyloid formation and fibrillogenesis in AD patients. The results of this study show that ApoE is a good biomarker for Alzheimer's disease. ApoE is also a good therapeutic candidate for the treatment of AD. ApoE may be a good target for the development of new therapies for AD.

ALZHEIMER'S DISEASE is characterized by senile plaques formed mainly from beta-amyloid (Aβ) protein. Genetic analyses have revealed an allele specific association of apolipoprotein E (apoE) isoforms with the relative risk for Alzheimer's disease. In order to investigate the possible existence of an apoE isoform-specific effect on the clearance of Aβ, synthetic Aβ-40 was labeled by iodination and incubated with Neuro-2a or RAW264 cells in the presence of human apoE4 or -e4 containing medium or control conditioned medium. After 4 h incubation at 37°C, cells were washed and treated with trypsin to remove surface bound Aβ. The trypsin resistant, cell-associated Aβ was quantified by measuring the radioactivity associated with the cells. When Neuro-2a or RAW264 cells were used together with conditioned medium prepared from stably transfected CHO cells, the cell-associated Aβ in the presence of apoE4 was >30% lower than that which cell-associated in the presence of apoE3. The results showed that apoE3 can have an isoform-specific effect on the association of Aβ with cells. The different patterns of ApoE association in these two cell types suggest that cell type-dependent factors are also involved in this process. The presence of apoE4 conditioned medium was >10% higher than that which cell-associated in the presence of apoE4 conditioned medium (p < 0.05). These results suggest that apoE4 may have an isoform-specific effect on the association of Aβ with cells. The different patterns of ApoE association in these two cell types suggest that cell type-dependent factors are also involved in this process.

APOLIPOPROTEIN E ALLELES IN CEREBRAL AMYLOID ANGIOPATHY AND INTRACEREBRAL HEMORRHAGE ASSOCIATED WITH ALZHEIMER'S DISEASE (AD). J. N. Kalantria, J. L. Cohen, and J. R. Reardon, Departments of Neurology and Pathology, Case Western Reserve University and University of Cincinnati Center, Cleveland, Ohio 44106, USA.

The presence of apolipoprotein E (APOE) - E4 allele has been implicated as a risk factor for AD. The association between the occurrence of APOE e4 alleles in AD associated with cerebral amyloid angiopathy (CAA) and other vascular lesions including intracerebral hemorrhage. We found significantly high frequency of the APOE e4 allele in AD subjects exhibiting moderate to severe CAA. This association was further corroborated in three other non-AD subjects with severe CAA in the absence of significant neocortical amyloid deposition, who were all homozygous for APOE e4 allele. Compared to all AD group, the frequency of the allele was also higher in AD cases with other vascular lesions such as multiple infarcts, ischemic white matter lesions, and petechial and subarachnoid hemorrhages. As previously reported, we confirmed that the frequency of the APOE e4 allele in the diffuse Lewy body variant of AD is intermediate between controls and AD subjects. Our results suggest APOE e4 allele to be a significant factor in the development of CAA in AD. The possibility exists that APOE may be a specific factor in vascular abnormalities associated with AD. Supported by grants from NIA and ADRDA.

BIRDS-AMYLOID: "B" by mechanically stable peptide outgrowth. The presence of ApoE in AD increases the risk of Alzheimer's disease. The presence of ApoE in AD is associated with an increased frequency of the apolipoprotein E (Aplo) e4 allele. Apolipoprotein E is a secreted glycoprotein involved in cholesterol transport and systemic outgrowth. AD, ApoE is also found in amyloid deposits. Apolipoprotein E binding to Aβ could lead to their aggregation into amyloid fibrils. Conversely, Apolipoprotein E allele may be protective against AD by inhibiting amyloid deposition in brains of AD patients at different stages of amyloid deposition including early and late dementia and 40-semester cases. Apolipoprotein E allele may be protected against AD by inhibiting amyloid formation and fibrillogenesis in AD patients. The results of this study show that ApoE is a good biomarker for Alzheimer's disease. ApoE is also a good therapeutic candidate for the treatment of AD. ApoE may be a good target for the development of new therapies for AD.


The major component of senile plaques in Alzheimer's disease is beta-amyloid (Aβ) which has neurotoxic properties and may contribute to neurodegeneration in AD. Another risk allele of ApoE, the e4 allele, has been associated with an increased risk of AD. Some studies have suggested that ApoE e4 allele may be associated with a reduced risk of AD. The present study examined the possible effect of ApoE e4 on the expression of Aβ and its receptor. Aβ was incubated with or without ApoE e4 for 1 h at 37°C. The incubation was then monitored for 1 h at 37°C. The Aβ was then removed and the supernatant was assayed for Aβ and phospholipase C activity. The results showed that ApoE e4 does not enhance the effect of Aβ on phospholipase C activity. The results also showed that ApoE e4 does not enhance the effect of Aβ on cellular calcium signaling.


In humans, apolipoprotein (apo) has three major isoforms, E2 (Cys3, Tyr23, E4 (Cys4, Arg24), and E4 (Arg4, Arg5), Arg6). ApoE is a genetic risk factor for Alzheimer's disease (AD). Recent evidence suggests that apoE2 or apoE4 may play a role in AD pathogenesis. It has been demonstrated that native preparations of apoE3 from conditioned media or plasma bind to AB with 20-fold greater avidity than apoE4. This preferential binding of AB to apoE3 is mediated by a binding process which increases delipidation and denaturation. Here we expand these observations to include AB binding to native apoE2, the isoform deficient in binding to AB in AD. To facilitate this study, we developed an ELISA to measure the binding of AB to apoE isoforms. The results showed that apoE2 and apoE4 bind to AB with higher avidity than apoE3. These results suggest that apoE2 and apoE4 may play a role in the pathogenesis of AD.
BETA-AMYLOID: ApoE II

670.15 EFFECT OF APOLIPOPROTEIN E ISOMERS ON B-AMYLOID-INDUCED TOXICITY IN RAT PRIMARY HIPPOCAMPAL CULTURES. M. T. Falduto*, D.L. LaDu, A.M. Manelli, G.S. Getz, and P.S. Puttarkcenk. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

Apolipoprotein (apoE), particularly the e4 allele, is genetically linked to Alzheimer’s disease. Immunoabsorption colonizes apoE with b-amyloid (Aβ) to serve plaques. In vitro, Aβ has been shown to be neurotoxic. While the role of apoE in the pathogenesis of the Aβ is unknown, one possibility is that it protects against Aβ-induced neurotoxicity. As the hippocampus is the brain region most affected by Aβ, we evaluated the effect of both native and purified preparations of apoE3 and apoE4 on Aβ-induced toxicity in primary cultures of rat hippocampal neurons. Morphological and biochemical changes were assessed following plating with apoE (30 µg/ml) or 15 µM Aβ(1-42). We have previously observed that apoE in conditioned media from HEK cells stably transfected with human apoE3 or apoE4 cDNA is lipid-associated. Using this native preparation as the source of apoE, both apoE isoforms protected against Aβ-induced neurotoxicity in primary rat hippocampal cultures. Toxicity was assayed by neurite length and cell viability (MTT assay) measurements made 3 days after exposure to the peptide. These results correlated with microscopic examination. Using purified apoE, again both isoforms protected against Aβ-induced toxicity. The addition of excessive b-migrating very low density lipoprotein (40 µg cholesterol/ml) alone did not provide protection against Aβ-induced toxicity or enhance the protection provided by purified apoE3 and apoE4 alone. These data suggest a role for apoE in protecting against Aβ-induced neurotoxicity.

670.17 EXPRESSION AND CHARACTERIZATION OF ALL THREE APOLIPOPROTEIN E ISOSFORMS (E2, E3, AND E4) IN INSECT CELLS. W.J. Chesnovich* and T. Burke. PanVera Corporation, Madison, WI 53711.

Apolipoprotein (Apo-) E is thought to play a role in the progression of Alzheimer’s Disease by way of its lipid transport role or virtue of its interaction with either b-amyloid protein, the microtubule protein tau, or both. There are three primary Apo-E isoforms, E2, E3, and E4, which differ from each other in one or two critical amino acids. We have purified all three human Apo-E isoforms after baculovirus-mediated expression in insect cells. Human recombinant Apo-E (hrApo-E) has an apparent molecular weight of 34 kDa. Two-dimensional gel electrophoresis reveals a complicated isofrom pattern which is reminiscent of the human serum Apo E pattern; along with the primary hrApo-E band, three-to-four additional bands occur at more acidic isoelectric points (pI), possibly representing sialylated and deamidated forms of Apo-E. The pI’s of hrApo-E2, E3, and E4 are 6.25, 6.35, and 6.7 respectively. hrApo-E, reconstituted in DMPC (dimyristoylphosphatidylcholine) liposomes, competes with 125I-LDL for binding to the LDL receptor. All three isoforms also bind to b-amylloid protein and tau.

BETA-AMYLOID: CELLULAR EFFECTS I

671.1 A PROTEIN KINASE CASCADE STIMULATED BY Aβ PEPTIDES: PKC, FAK AND FYN C. Zhang*, O. Krali, and W. Klein. Dept. of Neurobiol. & Physiol., Northwestern University, Evanston, IL 60208

It has recently been discovered that neuronal responses to Aβ peptides include a selective change in signal transduction associated with protein tyrosine phosphorylation (Zhang et al., J. Biol. Chem., 1994, 269:25247). This change is most evident in FAK, an unusual protein tyrosine kinase (Clark and Ibragim, 1995, Science 268:213) coupled to integrin and other receptors, to actin regulation, and to the ras signaling pathway. With respect to dose, kinetics, dependence on cell differentiation and requirement for Aβ aggregation, the evoked FAK Tyr (P) correlates with Aβ neurotoxicity, in both rat and human nerve cell lines. FAK thus provides an interesting molecular landmark to elucidate the cascade of cannabinoid events triggered by Aβ aggregates. We now show that aggregates of Aβ stimulate FAK-fos association, indicating that the Aβ-stimulation of FAK Tyr(P) is of functional consequence, and that PKC participates upstream in the signaling pathway from Aβ to FAK Tyr(P). How the cascade is triggered is unknown, although stimulation of FAK Tyr(P) by A3187 and by maz3 suggest that Aβ could cross the cascade by promoting Ca++/Ca++- or PKC-dependent stimulation (f-or immersion). While their potential dose-response consequences are extensive, the coupling of PKC, FAK and f- to cannabinoid pathways is especially intriguing, recent reviews (Hein, TINS 1993, 18:157) have suggested that ectopic entry to the cell cycle in neurons could cause profound synaptic immaturity significantly increased in Alzheimer’s-affected neurons (Shiotsi, Neuroreport 1993, 4:1435), this cascade could be a significant factor in Alzheimer’s pathogenesis. The cellular amyloid-evoked discontinuation of f-activity should prove to be neurodegenerative.

671.2 SECRETED FORM OF AMYLOID PRECURSOR PROTEIN PRIMES NAIVE PC12 CELLS FOR NEUROTOXIC EFFECTS OF NERVE GROWTH FACTOR. W.C. Wallace*, W.E. Abreu, C. Alber and V. Haroutunian. National Institute on Aging, NIMH, Baltimore, MD 21224, Mount Sinai School of Medicine, New York, NY 10029

Subcutaneous lesions of various neurotransmitter systems result in the in vivo induction and secretion of amyloid precursor protein (APP) in the corresponding projection fields of the cortex. In order to understand the physiological role of the secreted APP, we have studied the site of the induced APP in the brain. We have observed a dramatic increase in APP levels on day 12 and day 14 in the cortex. This increase is most dramatic in the neocortex, which is the site of the injection. This increase was observed following exposure to APP (1 ng/ml) and APP (15 ng/ml) as well as APP (1-5 ng/ml). None of these treatments produced significant changes in cell viability. These results demonstrate that this system is suitable for investigating the role of APP in the CNS.

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671.3 BETA-AMYLOID: JUNCTIONS BIOCHEMICAL APPROXIMATELY INCREASED T.

671.4 PHYSIOLOGIC LEVELS OF 8-AMYLOID (A8) ACTIVATE P3-KINASE AND PROMOTE NEURITE OUTGROWTH IN PC12 CELLS. Y. Lu; T. Sunderland and B. Wolozin Section on Geriatric Psychiatry, NIMH/NIH, Bethesda, MD 20892-1254

The A8 peptide plays an important role in Alzheimer's disease but is also present in blood serum and cerebrospinal fluid at 255 - 625 pM and has the potential physiologic actions of low level A8 have not been explored. We have found that picomolar doses of A8 can stimulate tyrosine phosphorylation of neuronal cells. One of the phosphoprotein proteins to A8 is phosphotyrosine/kinase (P3 kinase). Three independent lines of evidence support the hypothesis that A8 is activating P3 kinase through a tyrosine kinase mediated mechanism. Immunoblotting studies show that A8 induces tyrosine phosphorylation of p85 as well as binding of the p65 subunit of P3 kinase to tyrosine phosphorylated proteins. Studies of glycosylated membrane proteins show that A8 induces a translocation of p85 to membrane bound proteins, which are likely to be receptors. Finally, direct determination of lipid kinase products show that A8 increases the activity of P3 kinase. Activation of P3 kinase by A8 also stimulates PC12 cells to send out processes. Processes are evident within 6 hrs of application of 10 - 1000 pM A8; doses above this inhibit process outgrowth. Wortmann, a selective inhibitor of P3 kinase, blocks this response. Thus, physiologic levels at A8 stimulate tyrosine phosphorylation which leads to the activation of P3 kinase and stimulation of neurite outgrowth.


Previous studies have shown that PC12 cells overexpressing A8 amyloid peptide display altered morphology characterized by extensive intercellular appositions. To determine if these features are accompanied by gap junctional communication, we examined connexin43 (Cx43) in normal and A8-transfected PC12 cells. Normal and A8-transfected PC12 cells exhibited induced Cx43 expression by Western blotting, intracellular and plasma membrane-associated Cx43 in some cells of cultures processed by immunofluorescence, dye-transfer between some cells microinjected with Lucifer Yellow, gap junctions between cells examined by EM, and transmission of intercellular Ca
ter waves in cultures imaged with fura-2. Normal and vector-transfected PC12 cells exhibited none of these properties. Increased immunofluorescence in some clusters of A8-transfected cells was also observed with a monoclonal antibody against Cx32. Comparisons of Cx43 in normal and Alzheimer's Disease (AD) brain indicated that cortical areas with normal A8 amyloid plaques contained increased levels of Cx43 and some plaques corresponded to sites of elevated Cx43 immunoreactivity. If A8 amyloid peptide causes aberrant gap junctional communication through induction or increased expression of Cx43 in cells, this may compromise cellular homeostasis and exacerbate pathological conditions in amyloid plaques.

671.6 8-AMYLID DECREASES ADHESION OF FIBROBLASTS IN CULTURE. R.E. McGregor* and I. Schneider. Dept. of Psychiatry and Human Behavior, Miriam Hospital and Brown University, Providence, RI 02906

Fibroblasts from Alzheimer's Disease (AD) patients demonstrate several abnormalities. These include reduced spreading, decreased adherence on plastic culture plates and absence of a 113-55 potassium channel. The channel defect was simulated in normal fibroblasts by the addition of A8 amyloid. To study the effects of A8 amyloid on the adherence of cultured mouse fibroblasts, cells were exposed to the 40 residue form under a variety of conditions. By acutely or chronically administering A8 amyloid to cultured cells, we were able to demonstrate that the protein interferes with normal cell adherence on a number of substrates. Rates of adhesion with acutely added A8 amyloid were decreased on the laminin derived peptide IKVAV and on collagen I but not on plastic alone. The effect was concentration dependent. A8 amyloid, when used as a substrate, decreased adherence. It is apparent that A8 amyloid can have deleterious effects even on non-neuronal cell types.

671.7 PHYSIOLOGIC LEVELS OF 8-AMYLID AUGMENT PLATELET AGGREGATION. B. Wolozin*, C. Jones, R. Dukoff, N. R. Shulman and T. Sunderland. Section on Geriatric Psychiatry, NIMH and Clinical Hematology Branch, NIDDK, Bethesda, MD 20892

A8 amyloid is constitutively secreted by many cells and is normally present in our blood and CSF at levels ranging from 255-625 pM. Although micromolar levels of aggregated A8 is toxic to neurons, the functions of soluble A8 is unknown. We now report that all appears to play an important physiologic role in augmenting platelet aggregation. Addition of 1 nM A8 to 40% to gel filtered platelets in Tyrode buffer increased the sensitivity of the platelets to ADP-induced aggregation approximately 2-fold. Addition of all alone, however, did not induce platelet aggregation, both ADP and fibrinogen were required, and RGDS, which blocks fibrinogen-integrin binding, prevented aggregation. The all peptide augmented ADP-induced aggregation at doses of all beginning at 100 nM, peaking at 1 nM A8 and evident up to 1 nM. The reverse arginine sequence and the arginine sequence were both inactive, while all-16 showed weak 25% enhancement of platelet aggregation. Addition of wortmannin, which inhibits PI3 kinase, reduced the effects of all on aggregation, suggesting that PI3 kinase is involved in the all-response. Biochemical studies show that all induces rapid increases in tyrosine phosphorylation of a protein at 180 KDa, which occurs even in the absence of ADP or fibrinogen, suggesting that this process is directly related to the production of A8 amyloid. By physiological plasma levels markedly potentiates platelet aggregation possibly by activating a specific tyrosine kinase-linked cellular receptor.

671.8 UBIQUITIN-DEPENDENT PROTEIN DEGRADATION IS INHIBITED BY AMYLID BETA-PROTEIN. L. Gregersen*, M. Perera, and D. Goldgaber. Dept. of Psychiatry and Behavioral Science, Sch. of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794-4. Dept. of Pharmacology, Mt. Sinai Medical Center, New York, NY 10029

Ubiquitin and ubiquitin conjugate immunoactivity is typically observed in neurofibrillary tangles and inclusion bodies in the brains of individual affected by neurodegenerative disorders such as Alzheimer's disease and Down's syndrome. In addition to ubiquitin, amyloid beta protein (A8) immunostaining is also detected in neurofibrillary tangles. We investigated the correlation between the presence of A8 and the accumulation of ubiquitin conjugates. In our in vivo studies using radiolabeled l-lysine as the substrate, we found that synthetized A8 inhibited ubiquitin-dependent degradation pathway. In the presence of A8, lysine-lysine conjugates were formally formed and subjected to degradation. However, their degradation was inhibited which is consistent with A8 affecting the multi-subunit 26S proteasome activity. We tested the catalytic subunits of the 20S proteasome and found that A8 selectively inhibited the chymotrypsin-like activity within the proteolytic complex. Inhibition of ubiquitin by A8 was also found to explain the accumulation of high levels of ubiquitin conjugates which are found in neurofibrillary tangles and inclusion bodies. Furthermore, these studies identify A8 as an inhibitor of the 26S proteasome, which, if occurs in vivo, has important physiological significance and consequences.
EXOGENOUS AMYLOID Aβ1-42 STIMULATES THE INTRACELLULAR ACCUMULATION OF NEWLY SYNTHESIZED, 4 kDA AMYLOID PEPTIDE IN THE HIPPOCAMPUS OF TRANSFERRIN DEFICIENT RATS


Our earlier report indicated that intravenous Aβ1-42 aggregates after the turn-over of APP to cause the accumulation of insoluble APP and amyloidogenic fragments. To determine whether this accumulation ultimately gives rise to amyloid, we investigated the insoluble fraction of Aβ1-42-treated cells for the presence of 4 kDA amyloids. 35S-labeled 4 kDA Aβ peptides were immunoprecipitated from the detergent insoluble fraction and analyzed by SDS-PAGE and autoradiography. The amyloid peptides were purified by size gel filtration and reverse phase column chromatography. Sequence analysis of purified peptide indicated that some of the newly synthesized peptides are 'tagged' as previously observed for the exogenously added peptide. These peptides were eluted as a broad peak by a reverse-phase HPLC; this chromatographic properties is very similar to the Aβ1-42. In addition, the purified 35S-labeled amyloid peptides form SDS-stable high molecular weight aggregates with synthetic Aβ1-42, suggesting that at least a fraction of these peptides extends to residue 42. Therefore, our results suggest the intracellular Aβ1-42 aggregates serve as a nucleus for the further accumulation of additional newly synthesized Aβ peptides. Supported by NIH AG05538 and NS31230.
671.15 BETA-AMYLOID INHIBITS PROTEOLYSIS OF TAU PROTEINS BY RABBIT RETICULOCYTE LYSATE


Neurodegeneration in Alzheimer’s disease is accompanied by the accumulation of insoluble aggregates of tau proteins conjugated to ubiquitin. The DEAE-cellulose binding fraction of rabbit reticulocytes, containing enzymes of the ubiquitin degradation system (Fraction II) was used to conjugate bovine ubiquitin to tau proteins prepared from twicedigested bovine brain microtubules. In the presence of ATP, an ATP regenerating system, and hemin, which inhibits the breakdown of ubiquitin conjugates, high molecular weight ubiquitin-conjugated tau proteins accumulated. Tau-ubiquitin conjugation did not occur in the absence of ATP. In the absence of hemin, tau-ubiquitin conjugate and the tau proteins were degraded in an ATP- and ubiquitin-dependent fashion. Some tau proteolysis occurred in the absence of ATP; quantitative studies using [3H]labelled tau demonstrated that ATP-dependent proteolysis was 2-3 fold greater than ATP-independent proteolysis in this system. B-amyloid peptides inhibited the ATP-dependent proteolysis of 125-I labelled tau in the reticulocyte lysate system. Aβ(1-28) was a more potent inhibitor than Aβ(1-40). The effect of Aβ(1-28) was dose dependent and was half maximal at 5Pm peptide concentration. The degree of inhibition (40-70%) was comparable to that of 20 µM hemin. This system may provide an in vitro model of metabolic processes relevant to the pathogenesis of AD. Supported by the NIA (AG00504)

671.17amyloid beta peptide (25-35) INDUCES TAU PHOSPHORYLATION AND DECREASES MICROTUBULE-FORMING ABILITY IN RAT HIPPOCAMPAL CULTURE

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According to the amyloid hypothesis for the pathogenesis of AD, amyloid beta peptide (Aβ) directly affects neurons, leading to neurodegeneration and tau phosphorylation, followed by the production of paired helical filament (PHF) in neurofibrillary tangles (NFT). Consistent with this idea, primary cultures of embryonic rat hippocampal neurons undergo progressive degeneration as well as expression of an epitope for phosphorylated tau after exposure to Aβ. However, it is unclear whether the tau is phosphorylated at the same sites as PHF-tau, and whether the phosphorylated tau causes destabilization of microtubules in hippocampal culture after treatment with Aβ. These are crucial points for understanding the relationship between Aβ and phosphorylation of tau in the pathogenesis of AD. To address these points, we analyzed tau phosphorylation in a rat hippocampal culture treated with Aβ (25-35). By using antibodies that recognize phosphorylation sites of tau in a phosphorylation-dependent manner, we showed that the tau phosphorylation was enhanced in at least 5 sites. The phosphorylated tau accumulated in the cytoplasmic soluble fraction and showed reduced ability to support microtubule formation, as has been observed with PHF-tau. Thus, Aβ exposure could modify tau to a PHF-like state in hippocampal culture, suggesting that this culture system may be a useful model for studying the pathogenesis of AD.

671.18 BETA-AMYLOID: CELLULAR EFFECTS II

AB IMPAIRS ION-MOTIVE ATPase ACTIVITIES: EVIDENCE FOR A ROLE IN LOSS OF NEURONAL Ca2+ HOMEOSTASIS AND CELL DEATH

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The amyloid β-peptide (Aβ) that accumulates as insoluble plaques in the brain in Alzheimer’s disease (AD) can be directly neurotoxic and can increase neuronal vulnerability to anesthetic agents. We have previously shown that the α2-subunit of the Na+/K+-ATPase, Aβ, and subunits of the Na+/Ca2+ exchanger (ECa), both of which may be involved in the pathogenesis of Alzheimer’s disease. We recently showed that α2-subunit of the Na+/K+-ATPase is expressed in the brain and that Aβ impairs the activity of α2-subunit of the Na+/K+-ATPase. These results suggest that Aβ may contribute to the pathogenesis of Alzheimer’s disease.

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

BETA-AMYLOID: CELLULAR EFFECTS II

Maintenance of intracellular ATP is critical to neuronal survival during sublethal toxic insults induced by a wide variety of mediators generated during inflammation in the CNS in Alzheimer's disease. We investigated the role of extracellular ATP in the release of toxic mediators.

672.5 Depolarization of Mitochondrial Membrane Potential Accompanied β-amyloid-induced loss of mitochondrial cell viability, and with experimental protocol, even in vitro. Compromises in mitochondrial activity (redox state), which accompanies treatment with neurotoxic β-amyloid peptide (β-APP), is characterized by within 15 min in a variety of cell types, and precedes cell death in cultured rat hippocampal neurons. The redox compromise is associated with dose-dependent alterations in the pattern of depolarization of MPT product (N-acetylcysteine) in each cell. The subcellular distribution of the MPT product appeared to colocalize with a monocarboxyl mitochondrial dye in untreated cells. Since the cell membrane alteration is in all cases, it suggests that each cell possesses a component of redox which is sensitive to these peptides. We now report that β-A4 also produces a rapid depolarization of mitochondrial membrane potential, as measured with the fluorescent ratiometric probe MitoTracker Green FM (MTT), using confocal laser microscopy. This mitochondrial depolarization occurs in parallel with the compromise in MPT-reporting redox state. These data support an early mitochondrial involvement in a cellular response to β-A4, at concentrations which are selectively toxic to neurons.

672.7 β-AMYLOID INTERACTS WITH THE INTACT RECEPTOR MAC-1 RESULTING IN NITRIC OXIDE RELEASE FROM CULTURED MICROGLIA. L. Goodship, C. Martin, and E. Uyemura, Department of Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011.

While the β-amyloid protein of Alzheimer's disease (AD) has been well characterized biochemically, its primary biological function and mode of action in cells of interest in AD has not been determined. In previous studies, β-amyloid (β25-35 and β1-40) with IFN-γ was shown to be a major source of release of proinflammatory cytokines (IL-1β, IL-6, IL-12, TNF-α) from cultured microglial cells. A potential receptor for this effect is the microglial β2 integrin, Mac-1. We examined the interaction of β-amyloid (β25-35) with Mac-1 by a performing competitive binding studies between biotinylated β25-35 and FITC-labeled anti-Mac-1 antibodies to Mac-1 present in immunoprecipitates flow cytometry and b) observing the effects of anti-Mac-1 monoclonal antibodies (Mabs) on the microglial release of nitric oxide. For the competitive binding studies, microglial cells isolated from the hippocampal of β24-42 (Holczus) were exposed to biotinylated β25-35 (R-PE labeled), FITC-labeled anti-Mac-1 Mabs, or β25-35 followed by FITC-labeled anti-Mac-1 Mabs, and analyzed for fluorescence intensity by flow cytometry. For the nitric oxide studies, bartered microglia were plated onto a polystyrene surface and exposed to β25-35 in the presence or absence of anti-Mac-1 Mabs, incubated for 24 hour and analyzed for nitric release. Flow cytometry revealed β-amyloid binding and upregulation of anti-Mac-1 Mac binding to microglial cells. Cultured microglia showed a decrease in β-amyloid/IFN-γ induced NO release when treated with anti-Mac-1 Mabs. Our study suggests an interaction between β-amyloid and the microglial Mac-1 receptor resulting in NO release. Such an interaction may have implications in the neurodegeneration seen in Alzheimer's disease.

672.8 AMYLLOID PRECURSOR PROTEIN INHIBITS ASCORBATE INDUCED LIPID PEROXIDATION IN HUMAN CORTEX. A.C. Andersen and M.A. Pappolla, Dept. of Psych., St. Louis University, St. Louis, MO (63110) and Dept. of Pathol., Univ. of Texas Health Sci. Cent., Houston, TX (77030).

Amyloid precursor protein (APP) is the progenitor of β-amyloid (βA) which accumulates in fibrillar form in neurotrophic plaques and walls of blood vessels in Alzheimer's disease (AD). Lipid peroxidation (LP) is a result of increased oxidative damage which may play a role in the pathogenesis of AD. The interaction between APP and LP was not known. We now report that when ascorbate (5.1 mM) is used to stimulate LP in particular membrane fragments derived from cynomolgus normal human prefrontal cortex, APP completely inhibits the stimulated LP. Computer assisted analysis of the merged dose-response data showed an IC50 of 5.7 ± 10-6 M. Similar analysis of data obtained using fragments of APP, showed the following IC50: BSA-25-35 (2.4 ± 10-5 M), βA (β25-35) (3.4 ± 10-5 M), βA that had been incubated to produce the neurotoxic equivalent and then used in the experiments generated an IC50 of 3.6 ± 10-5 M. These data suggest that APP and some of its cleavage products can prevent LP at certain concentrations. The physiologic relevance of this finding remains to be determined.
**672.9 FREE RADICAL INVOLVEMENT IN β-AMYLOID TOXICITY.** C. Cali, G. Tosi, 1 Tinetti, N. Angiulli, E. Luca, G. Fortini, E. Marrazzo.

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Previous data showed that a synthetic peptide (8-25,35) homologous to residues 25-35 of β-amyloid induced neuronal death by apoptosis. There is some evidence that oxygen radical reactions play a role in Alzheimer's disease pathophysiology. Furthermore, oxidative stress has been shown to be able to induce neuronal cell death mechanisms. In this study, we have focused on the role of free radical reactions may play a primary role in neurodegenerative events associated with AD development. Primary cortical neurons were exposed chronically and acutely to 50 μM 8-25,35 for the acute exposure a solution of the peptide was added to the medium after 7 days of culture and the effect was observed the following day. For the chronic treatment condition was a simple of constant 3 days for 3 days or repeated treatments every 2 days up to day 7 in culture.

The following parameters were evaluated: antioxidant enzymes superoxide dismutase and glutathione peroxidase, lipid peroxidation products (TBARS) and intracellular reactive oxygen species production, using a fluorescent probe, dichlorofluorescein diacetate.

8-35,25 treatment induced a marked increase of superoxide dismutase activity only after acute exposure, while it had no effect during chronic treatment. Glutathione peroxidase showed changes only following chronic treatment. Lipid peroxidation index was increased during chronic treatment, while the acute exposure of neurons to 8-25,35 showed a proapoptotic protective effect, being lipid peroxidation product level significantly higher in the control neurons. ROS production, measured fluorimetrically, was markedly enhanced in both chronically and acutely exposed neurons. In conclusion, our results suggest that oxidative stress and free radical production are somehow linked to the peptide presence, and, may be, to its neurotoxicity.

**672.11 EVIDENCE FOR DNA DAMAGE, BUT NOT APOTOPSIS, IN ALZHEIMER'S DISEASE (AD) BRAIN.** L.S. Perlmutter,1 A.F. Bushnell,1 P. Li,2 S.F. Wohl,1 J. Geurts,1 M. Cappai,1 E. Gasser,1 D. Tillakaratne,2 R. Munsat,2 J.J. Estus3,4 and J.B. Perry.1 1 Institute of Neurology, National Hospital, London, UK. 2 Institute of Biomedical Science, University of Vienna, Vienna, Austria. 3 Istituto di Ricerca Scientifico Mario Negri, Milan, Italy. 4 Unit of Neuropathology, New York, USA.

We have previously identified a pattern of gene expression that accompanies the neuronal apoptosis of rat sympathetic neurons induced by NGF deprivation. This temporal cascade begins with transcription factors such as c-jun and c-myb, followed by c-fos, fos β, NGF7-41, and rh, and then two extracellular matrix proteases regulated by NGF and c-fos, i.e., trans-1 and collagenase. Moreover, neutralizing antibodies against the Jun family, the Fos family, or c-jun specifically, blocked apoptosis, indicating that these gene products are necessary for apoptosis. To examine whether this genetic cascade occurs in other in vitro models of neuronal death, we have examined rat cortical neurons (i) treated with the Aβ (1-42) or (ii) subjected to glucose deprivation. When neurons were treated with 20 μM Aβ, we observed robust induction of c-jun, beginning at 12 hours, followed by c-fos and fos β, beginning at 24 hours, with death (LDH release) beginning at 48 hours. Similar genetic changes were not observed after glucose deprivation. In future experimentation, we shall confirm and extend these findings. The present results suggest that the induction of c-jun and c-fos may be specific to certain types of neuronal death.


Aggregated β amyloid peptides induce neuronal death in cultures. Although the mechanism of this death is still unknown, proposed possibilities include: i) formation of Ca2+ ionophores; ii) destabilization of Ca2+ homeostasis; and iii) generation of free radicals.

Murine cortical cell cultures exposed to 10-40 μM synthetic β amyloid (β1-42; obtained from K-biologicals) developed concentration-dependently, gradually occurring neuronal death, partially blockable by the potent antioxidant cycloheximide. This β1-42 induced neuronal death was not associated with an increase in neuronal [Ca2+], measured at 12 and 24 hr after exposure onset. Indeed, it was potentiated by lowering extracellular Ca2+, or by variation of glutamate agonists (10 μM MK-801 or 50 μM CNQX), or 20 μM nimodipine. Furthermore addition of the phorbol ester PKC activator, 10 nM PMA, or 30 nM UK, reduced β1-42 induced neuronal death. Addition of rotenone, superoxide dismutase, or α-lipoic acid, did not attenuate the β1-42 neurotoxicity.

These data are consistent with an idea that β1-42 induced neuronal death may occur at least in part via mechanisms involving intracellular Ca2+ insufficiency and new macromolecule synthesis, and fit with other data suggesting that this death may involve apoptosis (Loo et al., FNAS 90, 1991). Supported by NIH NINDS grant NS 30337 (DWC).

**672.13 COMPARISON OF ALTERED GENE EXPRESSION DURING NEURONAL DEATH INDUCED BY NGF WITHDRAWAL, AMYLOID β-PROTEIN TREATMENT, OR GLUCOSE DEPRIVATION.** S. Santin*, C. Gai, G.C. Rovarelli, M. Mattacca, I.F. Brooksam, and E.F. Rydel*.

1 Depts. of Physiology, Neurobiology and Anatomy, Sanders-Brown Center, University of Kentucky, Lexington, KY 40536, and Athena Neuroscience, Inc., San Diego, CA 92121, USA.

We previously identified a pattern of gene expression that accompanies the neuronal apoptosis of rat sympathetic neurons induced by NGF deprivation. This temporal cascade begins with transcription factors such as c-jun and c-myb, followed by c-fos, fos β, NGF7-41, and rh, and then two extracellular matrix proteases regulated by NGF and c-fos, i.e., trans-1 and collagenase. Moreover, neutralizing antibodies against the Jun family, the Fos family, or c-jun specifically, blocked apoptosis, indicating that these gene products are necessary for apoptosis. To examine whether this genetic cascade occurs in other in vitro models of neuronal death, we have examined rat cortical neurons (i) treated with the Aβ (1-42) or (ii) subjected to glucose deprivation. When neurons were treated with 20 μM Aβ, we observed robust induction of c-jun, beginning at 12 hours, followed by c-fos and fos β, beginning at 24 hours, with death (LDH release) beginning at 48 hours. Similar genetic changes were not observed after glucose deprivation. In future experimentation, we shall confirm and extend these findings. The present results suggest that the induction of c-jun and c-fos may be specific to certain types of neuronal death.


Cerebral amyloid-β protein deposits is a major pathological feature of Alzheimer's disease. Also in some sporadic encephalopathies (SE), altered form of prion protein (PrP) aggregates in amyloid fibrils and accumulates in the brain of affected individuals. Synthetic peptides homologous to β25-35 and to P10 106-126 fragment induced neuronal death by apoptosis or by pyroptosis, respectively. Furthermore, according with pathological features of SE, chronic treatment with PrP 106-126 increased the proliferation rate and CAFA expression in astrocytes β25-35 and P10 106-126 have a β-sheet structure and exhibit self-aggregation properties. Since the neurotoxicity of these peptides has been associated with their fibrillogenic activity, we synthesized amidated homologous, β 25-35 NH2, and P10 106-126 NH2. To obtain peptides with low level of amyloidogenic activity and directly test the relationship between amyloid fibrils and neuronal death or astrogial proliferation. The β-sheet conformation and the self-aggregation activity of both amidated peptides were strongly reduced as determined by circular dichroism spectroscopy analysis and electron microscopy examination. Rat hippocampal astrocytal cells were chronically exposed to both amidated peptides (25-100 μM). β25-35-NH2 2 compared to β 25-35 reduced its neurotoxic activity, while the neuronal cell death induced by P10 106-126-NH2 was simmilar to that observed in the β25-35. In contrast rat astrocytes chronically exposed to P10 106-126-NH2 (25-100 μM) did not alter their proliferation rate and CAFA expression compared to control condition was unchanged. Thus, astrocytosis induced by P10 106-126 appear associated with self-aggregation activity while toxic effect, unlike β25-35, was independent of the peptide amyloidogenic activity.
672.15

ANALYSIS OF TRANSCRIPTIONAL CHANGES IN RESPONSE TO β-AMYLIDO INDUCED NEUROTOXICITY. T. Sunderland, I. W. Kustak and R. Wolpaw. Section on Geriatric Psychiatry, NIMH and Molecular Neurobiology Unit, NIA, Bethesda, MD 20892.

Recent studies have shown that specific transcripts, including Jun and Fos, are upregulated in neuronal apoptosis (Freeman, et al., Neuron 12: 343-355, 1994). We have now analyzed changes in gene expression occurring during β-amyloid (Ab)-induced cell death in order to better understand the mechanism of action of aggregated Ab. Cortical neurons from E18 rats were grown in culture for 10 days, transferred to serum free medium and then treated with 10 μM Ab42 for 2-7 hrs. The RNA was harvested and transcriptional changes were analyzed by semi-quantitative PCR. Several transcripts examined, including GSPDH, NGF-IA and p53, showed no changes during the course of treatment with Ab. Two transcripts, Fox and Bel-X1, showed rapid and persistent elevation evident 4 hrs after application of all. The levels of Jun, Bax and cyclin D1 also increased, but over a slower time course, being evident 10-24 hrs after treatment with Ab. The levels of Bcl2 increased from 4-10 hrs after application of all, but then declined as the levels of Bax increased. This reciprocal expression of Bcl2 and Bax has been observed in other systems as well. The patterns of gene expression induced in response to aggregated all resemble that seen during neuronal apoptosis in response to growth factor withdrawal, and set the basis of an understanding of all-induced cell death on a molecular level.

672.16

GENERATION OF AMYLOGENIC ALZHEIMER'S β-AMYLOID PRECURSOR FRAGMENTS DURING H2O2 INDUCED APOPTOSIS IN HUMAN NEURONAL CELLS. Z. Zhang,1,2 I. W. Kustak,2 B. Zhang2 and G. S. Roth.1 Molecular Physiology & Genetics Section, Lab. of Cellular & Molecular Biology, Molecular Neurobiology Unit, Lab. of Biological Chemistry, NIA/NIH, Baltimore, MD 21224

A number of groups have reported apocitic cells in Alzheimer's brain. This suggests a role for neuronal programmed cell death (apoptosis) in the development of Alzheimer's disease (AD). To explore how apoptosis may relate to AD pathophysiology, we studied the processing of amyloid precursor protein (APP) during H2O2 induced apoptosis in cultured human embryonic stem cells. We found increased levels of amyloidogenic fragments of 12 and 16 kDa both prior to and after apparent induction of neuronal programmed cell death which was indicated by DNA laddering. The level of full length APP was dependent on the concentration of H2O2 used. We conclude that a neuronal apoptotic process may generate fragments containing intact Aβ42 peptide which is neurotoxic. Since βA4 has been recently shown to induce apoptosis, we further hypothesize that amyloidogenic fragments may induce a cycle of apoptosis which generates additional toxic fragments and such a vicious cycle may contribute to the neuronal loss during AD development.

ALZHEIMER'S DISEASE: MECHANISMS OF DEGENERATION I

673.1


The cytokine interleukin-1 (IL-1) is a potent neurotoxic factor that is present in elevated levels in brain of Alzheimer disease (AD). IL-1 induces excessive expression of α-amyloid precursor protein (APP), suggesting that IL-1 is an important pathogenic factor in plaque evolution. We used computer imaging to determine the number, size, and immunoreactive intensity of IL-1α immunoreactive (IL-1α-IR) microglia in temporal lobe of 9 Alzheimer and 4 control patients. The number of IL-1α-IR microglia and the average IL-1α-IR cell area in cortical layers 1 and 2 were similar in Alzheimer and control patients. In contrast, in layers 3 through 6, the number of IL-1α-IR microglia in Alzheimer's disease was significantly elevated when compared to controls (3.5-fold in layer 3 and 2-fold in layers 4, 5, and 6), as was the IL-1α immunoreactive content of microglia (2-fold higher in layers 4, 3, and 5; and 1.2-fold higher in layer 6; p < 0.05). The distribution of β-APP+ plaques in cerebral layers correlated with that of IL-1α-IR microglia (R = 0.99; P < 0.005), as did the total immunoreactive area of these β-APP+ plaques (R = 0.95; P < 0.05). This topographical correlation between microglial IL-1α and neuritic β-APP overexpression supports a role for IL-1α in the induction of β-APP overexpression and promotes the dystrophic neurite formation in Alzheimer's disease, thus in the evolution of diffuse amyloid deposits into neuritic β-amyloid plaques. Supported in part by NS27414, AG10208, and AG12411.

673.2

C1q IN ALZHEIMER'S DISEASE CORRELATES TO SEVERITY OF DEMENTIA AND COLOCALIZES WITH NATIVE PLAQUES AND NEURONS. N.C. Berchtold, B.J. Cummings, D.G. Gr, R. Sharkle, D. McCurry, A. Alagh, J. Olan, A.D. Tenner, G. Cutipag, Institute for Brain Aging and Dementia, Irvine, CA 92717-4550 USA.

Numerous studies have implicated the immune system as being contributing to the neuropathological process of AD. C1q is known to induce significant changes in AD progression. To be able to confirm the role of C1q as an immune system participant, we have developed a new monoclonal antibody that recognizes the native form of C1q and we have used this antibody to study C1q in AD postmortem brains. C1q appeared to be increased in AD brain and was not consistently found in the controls. We have also demonstrated that C1q co-localizes with AD plaques, as well as with AD neurons. We have therefore demonstrated that C1q plays a significant role in AD pathology, and we speculate that C1q may be a potential therapeutic target for AD.

673.3

EXPRESSION OF COMPLEMENT INHIBITORS C1 INHIBITOR AND PROTECTIN (CD59) BY HUMAN NEURONS DERIVED FROM INTERA TERA2TERATOMACARISMA CELLS. D.G. Walker,1 X. Xiao, R. E. McGarr and P.J. McGarr. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T2Z2.

Studies of brains from Alzheimer Disease (AD) cases have shown evidence of inflammatory changes in affected tissue, as demonstrated by the presence of reactive microglia and macrophages. The classical pathognomonic immunohistochemical lesion of the complement system in brains of AD patients may be leading to the death of healthy neurons (bystander lysis). Immunohistochemical studies from this laboratory have indicated the inability to express certain proteins that can inhibit different stages of the complement cascade. To explore further the potential expression by neurons of complement cascade inhibitory molecules, pure cultures of cells with the characteristics of post-mitotic human neurons were prepared from the INTERA2 teratocarcinoma cell line. Following ex vivo acid treatment, purified cells were prepared for gene expression studies by short term culture in serum-free media. Cells were co-cultured with y interferon, interleukin 1β or phorbol myristate acetate. Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, C1 inhibitor gene expression was detected in a number of independent post-mitotic neuronal cell cultures. C1 inhibitor expression was low or absent in unstimulated neurons, and was greatly increased by treatment of cells with y interferon. Proteins gene expression was detected in these cells by R-PCR, by immunoassay analysis and by immunocytochemistry. In contrast to C1 inhibitor, protection expression was not increased by y interferon stimulation. These results indicate that human neurons can express crucial complement inhibitory proteins. Understanding how these proteins can be regulated may lead to potential therapeutic strategies for affecting the progression of neurodegenerative diseases like AD.

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

673.4

INFLAMMATORY MECHANISMS OF NEURODEGENERATION. THE ROLE OF THE COMPLEMENT DERIVED ANAPHYLATOXIN C5a. T. Topol1, W. Weilbch, and O.M. Feinstadt2,1 Division of Neuroscience, Hedo Neuroscience Program, USC, Los Angeles, CA 90089; 2Division of Neurogerontology, Andrus Gerontology Center, USC, Los Angeles, CA 90095.

This study addresses the role of the proinflammatory anaphylatoxins C5a in mechanisms of hippocampal neurodegeneration. We found that the pyramidal neurons in the CA3 sub-region of the hippocampus form in congenital genetically deficient in complement component C3 (C5a) are more susceptible to glutamate mediated neurodegeneration, relative to C5-sufficient mice (C5a-). Moreover, the C3 derived anaphylatoxin C5a (human recombinant) has been shown to diminish glutamate-mediated neurotoxicity in vivo. Therefore, the proinflammatory anaphylatoxins C5a might also be a neuroprotectant. Potential mechanisms include regulation of glutamate receptors and local control of cytokine expression that may influence hippocampal responses to lesions. We found that C5a neutralizing mice show selective impairment of C5a dependent regulation of glutamate AMPA receptors in CA1/CA3 pyramidal neurons during response to hippocampal lesions and found that hippocampal astrocytes cultured from C5a mice have hypersecretion of the cytokines IL-6 and TNF. These data are consistent with our finding showing C5a receptor (C5aR) expression in CA1/CA3 pyramidal neurons and astrocytes of the mouse hippocampal formation. This study may lead to a better understanding of complement-mediated pathophysiology in Alzheimer disease and other neurodegenerative diseases which involve excitatory AAs pathways. Ongoing studies using a C5aR knockout mouse model will clarify the roles of the pro-inflammatory anaphylatoxins C5a in brain. This work was supported by the Nathan W. & Margaret T. Shock Aging Research Foundation to GMP.
673.5 Coexistence of Alzheimer's disease with Herpes virus Encephalitis. P. Staub, J. Colmer, P. Denaro and D. Freed. Texas Tech University HSC, Lubbock, TX 79430

At present there are no treatments for Alzheimer's disease but diagnostic techniques have progressed using peripheral markers and clinical tests. Problems in treatment or diagnosis can arise if Alzheimer's disease is present with other conditions, such as herpes virus encephalitis. In the present report we examine both the clinical progression of dementia and the histological picture of herpes virus superimposed on Alzheimer's disease. Several important points were noted: the mental status of Alzheimer's patients should be evaluated at routine intervals. If a sudden change in mental status occurs, evaluation of other medical conditions should be performed. With the advent of PCR, viral testing may be more easily done and antiviral treatment initiated if needed. Examination of the brain of this Alzheimer's patient revealed viral infection using EM, immunocytochemistry and in situ hybridization. In addition, the histological picture of AD was superimposed in some of the affected areas.

673.6 Mercury Vapor Inhalation Inhibits Binding of GTP to Tubulin in Rat Brain: A Molecular Lesion Present in Alzheimer Brain. F.L. Lonserheild, M.J. Virys, J.C. Pendergrass and B.E. Huleot. Univ. of Calgary Faculties of Med., Calgary, AB T2N 4N1 CAN and Univ. of Kentucky Coll. of Pharm., Lexington, KY 40505 USA.

HG interact with tubulin and disassemble microtubules that maintain neurite structure, and is continuously released from "silver" amalgam tooth fillings and is absorbed into brain (FASEB J. 9: 504-508, 1995). In the present study rats were exposed to HG4 h/day for 0.2, 7, and 28 at ~250 mg HG/ml air, a concentration present in mouth air of some humans with many amalgam fillings. Average rat brain HG concentrations increased significantly (11-47 fold) with duration of HG exposure. By 14 d HG exposure, photoinactivity labelling of the subunit of the tubulin dimer with [3F]-PNI in brain homogenates was decreased 75-75%, upon analysis of SS-PSAGE autoradograms. The identical neuronal lesion of similar magnitude is evident in most Alzheimer brain homogenates when compared to human age-matched controls. Since the rate of tubulin polymerization is dependent upon binding of GTP to tubulin, we conclude that chronic inhalation of low-level HG can inhibit polymerization of tubulin essential for formation of microtubules. (Supported by: NIH GM35766; Wallace Genetic Fnd; IAOMT)

673.7 An Inhibitor of the 20S Proteasome Increases a Stress Response in a Mouse Neuronal Cell Line, Manifested by Accumulation of Ubiquitylated Proteins and OF the Induced Hsp70. Sophie Kaudiel & Ronald P. Magnusson, Dept. of Pharmacology, Mount Sinai School of Medicine of CUNY, N.Y., N.Y. 10029.

Ubiquitin-protein conjugates are commonly detected in neuronal brain inclusions of patients with neurodegenerative diseases suggesting that a common molecular response involving ubiquitylated proteins occurs with all neurodegenerative diseases. The ubiquitous ATP-dependent proteolytic system (20S proteasome) plays a major role in the removal of short-lived, abnormal and denatured proteins. The catalytic core of the 20S proteasome is the multicatalytic protease complex (MCP) or 20S proteasome. Recently, we showed that exposure of HT22 cells (a mouse neuronal cell line) to 25 μM of α-thiosemicarbazide (an irreversible inhibitor of MTC (26S-DDB)AL-CHD) induced the accumulation of ubiquitylated proteins. We now show that overnight incubations with concentrations of the same MCP-inhibitor as low as 0.25 μM also produce accumulation of ubiquitylated proteins. The ladder-like pattern and increased intensity of the immunostaining observed in Western Blots of total cell extracts probed with an antibody against ubiquitin protein conjugates depicts an increase in the accumulation of ubiquitylated proteins as compared with that observed after shorter (3h) incubations. Overnight incubations with ubiquitin-conjugated α-thiosemicarbazide (ZL-CHD) produced no accumulation of ubiquitin-protein conjugates. Furthermore, overnight incubations with the MCP-inhibitor but not with the calfaparin, led to accumulation of the inducible form of the heat shock protein HSP70 in HT22 cells. Northern Blots showed that there was a concentration dependent increase in HSP70 mRNA coexisting with the increase in protein expression detected by Western blots probed with a specific antibody for HSP70. These results suggest that modification of the 20S proteasome resulting from a decrease in MCP activity, induces a stress response in the neuronal cells, accumulation of ubiquitylated proteins, and ultimately may lead to neuronal death. This mechanism may be involved in the etiology of neurodegenerative diseases. (Supported by NIH grant NS29396).


In many neurological and neurodegenerative disorders, neuronal death can result from the excessive release of endogenous excitatory amino acids like glutamate. As excitotoxicity may constitute a final common pathway for neuronal injury and death, we investigated the dynamics of the toxic effect of another endogenous substance, quinolinic acid, after its injection in the medial septal/diagonal band area (MS/DB) of L-Evans male rats.

Quinolinic acid (60, 180 and 600 μl in 0.5 μl) was injected into the MS/DB area, and histological, biochemical as well as behavioral studies were conducted starting 3 days after injections. Groups of rats were killed 3h, 24h, 7d and 14d. Lesioned (L) rats were also included for comparison. Histological evaluation after hematoxylin-eosin staining of paraffin-embedded sections revealed a differential degree of necrosis, gliosis proliferation and gliosis formation in the MS/DB area. The extent of neuronal and microglial necrosis in the hippocampus was present only in quinolinic injected animals and appeared to be time-dependent and dosage dependent. In the behavioral studies, quinolinic acid induced a dose-dependent aversive stimuli in the elevated plus maze model of anxiety. In the water maze test, an impairment in the processing of spatial information was present without any effect on cue learning. Hippocampal atrophy was significantly reduced only in 24 h lesioned rats. The neurodegenerative process induced by quinolinic acid injections in the MS/DB area indicates the sensitivity of the septo-hippocampal neurons to this endogenous excitotoxin as demonstrated by histological and behavioral parameters.

673.10 Effects of a Bilateral Neurotoxic Lesion of the RHINAL CORTEX on Cerebral Glucose Utilization (CMRglc) in Baboons: A PET STUDY. K. Megan, C. Chav Icons, K. Bivock, C. Le Menicott, P. Hansen, E.T. Mackenzie* and J.C. Baroc. INSEAN U320, CNRS URA 1629, Centre Cynopere, University of Caen, BP 5229, 14074 Caen, France. Ablations in the rhinal cortex may play a crucial role in Alzheimer's disease (AD). In the AD, the entorhinal cortex (ERC) is, with hippocampus, the area most affected by neurofibrillary tangles with the cerebral metabolic ratio for glucose is most reduced in temporal-occipital association cortex and to a lesser extent in temporal cortices. This feature has been used as a marker for the severity of dementia. Furthermore, combined ablations of ERC and parietal cortex (PCR) in macaques leads to a severe disruptive memory deficit. To assess the relationships between rhinal alterations and changes in cerebral metabolism we studied the effects of stereotaxic bilateral lesions of ERC and PCR on CMRglc in Papio anubis baboons. Magnetic resonance imaging (MRI) was used to identify the target sites (n=21-22 per site). A stereotaxic frame was used to coregister the MRI to the actual surgical template and to identify brain structures for CMRglc measurements. Cortical PET coregistered with MRI was performed under propofol anaesthesia (10 mg kg⁻¹ h⁻¹) for 30 minutes or 30-60 minutes depending on the animal. Cerebral blood flow was assessed using F-15O H₂ and O-15 H₂O inhalation to a target level of 14% above baseline. In order to simulate intellectually normal baboons. The areas most affected were not only the hippocampus but also the temporal, occipital associative and insular cortices, consistent with the known neurological connections of the rhinal areas. Declines in CMRglc more or less important than expected (e.g. in basal ganglia and cingulate, respectively) also occurred. These preliminary results suggest that severe rhinal neuronal loss (histologically confirmed in two baboons) induces a long-lasting effect on brain metabolism. Relationships between these metabolic effects and memory function are currently being investigated.

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673.11
NON-A) COMPONENT OF ALZHEIMER’S DISEASE AMYLOID (NAC) IS ALZHEIMODIGENIC. A. Iwaw*, M. Yoshimoto, E. Masliah and T. Sallow, Department of Neurosciences 0654, University of California at San Diego, La Jolla, CA 92039/0624

The non-A) component of Alzheimer’s disease (AD) amyloid (NAC) was identified chemically as the second major component in the amyloid purified from brain tissue of AD patients. NAC, derived from its 140-amino-acid long precursor, NACP, is at least 36 amino acids long (NACP36) although its amino terminus is not definitively determined. Affinity-purified anti-NACP-X1 antibody against the amino-terminal 9-amino acid sequence of NACP36 immunostained amyloid in AD brain sections and recognized NACP36 but not NACP on Western blots. In aqueous solutions, synthetic NACP36 self-aggregated in a time-, concentration-, and temperature-dependent manner. NACP35 was detected initially as a monomer with a molecular mass of 3600 Da, but became aggregated as a function of time into a higher molecular mass component that could not migrate into the gel. The aggregate of NACP35 showed green-gold birefringence after Congo-red staining when analyzed under polarized light and a fibrous-like structure when analyzed ultrastructurally. These results suggest that NAC can form amyloid after it has been cleaved out of its precursor and may be a crucial factor in amyloidosis in the brain. This work has been funded by Alzheimer’s Disease Research, a program of the American Health Assistance Foundation, NIH grant AG05131, and was supported by Yamanouchi Pharmaceutical Co., Ltd.

673.13

Alzheimer’s disease (AD) is associated with the accumulation of B-amyloid protein and the loss of specific neurons. B-amyloid has been shown to induce neuronal apoptosis in vitro and has been suggested that apoptosis may be involved in Alzheimer’s related neurodegeneration. Apoptosis appears to be regulated by the ratio of bcl-2, which promotes cell survival, and bax, which accelerates cell death. Both of these genes are present in neural tissue. We sought to investigate the possibility that changes in the expression of the bax protein in the aged brain may contribute to the progression of Alzheimer’s related neurodegeneration. Therefore, we examined the immunohistochemical distribution of bax protein from samples of brain tissue from control and Alzheimer’s disease patients fixed with formaldehyde and severe and AD. Sections were blocked with 4% formalin, preincubated with a polyclonal antibody specific for bax (Santa Cruz) or preabsorbed with the immunizing peptide. Following incubation with biotinylated goat anti-rabbit IgG, sections were incubated with avidin-biotin peroxidase complex and diamobenzidine was used as a chromogen. Immunoreactive bax protein was detected within the cytoplasm of a small population of neurons. Additionally, bax immunoreactivity was detected within the epithelial cells of the choroid plexus. Glial cells did not appear to be immunoreactive for bax. The number of bax immunoreactive neurons appears to correlate with the severity of AD. These results suggest that the presence of bax protein may be associated with the progression and severity of AD. Supported by funds from AB1062 and Rhode Island Hospital.

673.15
PURIFICATION AND DETAILED CHARACTERIZATION OF PERLECAN ISOLATED FROM THE ENGELBREIT-HOLM-SWARM (EHS) TUMOR FOR USE IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE IN BRAIN. G.M. Castille, J.D. Miller, J.A. Cummings, C. Ngo, W. Yang and A.D. Soper*, Dept. of Pathology, Neuropathology Lab, Box 364600, University of Washington, Seattle, WA 98195-6480.

Previously we have demonstrated that infiltration of perlecan (a specific heparan sulfate proteoglycan) and beta-amyloid protein (Ab) into rat hippocampi leads to a consistent animal model to study the effects of fibrillar Ab amyloid in brain (Neuron 12:219-234, 1994). We have recently observed that the final purity of perlecan may be important to the observed effects in the animal model such that contaminating proteins produced by the EHS tumor may reduce the extent and consistency of congophilic Ab amyloid in brain. In the present study, we describe our method of purifying perlecan, and the stringent testing we employed to ensure the highest possible quality of our perlecan preparations for use in the animal model. Briefly, 50 or 100 grams of EHS tumor tissue is extracted using 4M guanidine-HCl by anion-exchange and gel filtration chromatography. SDS-PAGE followed by staining with silver and Coomassie blue, demonstrated no other contaminating proteins in our perlecan preparations. Western blots using a specific perlecan core protein antibody (H-K-102) showing heparanase digestion show a characteristic doublet at 400 and 360 kDa indicative of intact perlecan core protein. Absence of contamination by other basement membrane components produced by the EHS tumor is confirmed by absence of immunoreactive bands on Western blots using antibodies against laminin, fibronectin or collagen type IV. Our procedures for isolation and characterization of perlecan from EHS tumor ensures perlecan of the highest quality, to maximize the potential effects of Ab amyloid deposition/persistence in brain in the animal model.

673.16
DNA FRAGMENTATION IN ALZHEIMER’S DISEASE HIPPOCAMPUS: CORRELATION WITH TAU AND β-AMYLOID IMMUNOREACTIVITY. M. Dragowska, A. Anggono, R.L.M. Pauls, H. Waldvogel, Departments of Pharmacology and Anatomy, School of Medicine, Auckland Univ, Auckland, New Zealand.

A number of recent studies indicate that cells in postmortem brain tissue from Alzheimer’s disease cases show in situ evidence of DNA fragmentation, suggesting that these cells are undergoing apoptosis. We have examined hippocampal sections from neuropathologically-normal and Alzheimer’s disease post-mortem cases and compared DNA fragmentation (detected using the TUNEL method) with Tau and β-amyloid immunoreactivity. In addition, we performed double-label studies to investigate whether TUNEL-positive cells were also Tau, calbindin, calretinin, and/or GFAP positive. Results show that most TUNEL-positive cells in Alzheimer’s disease hippocampus are not neurons and there is no strong correlation, either positive or negative, observed between the number of TUNEL-positive cells and the number of Tau-positive cells. The implications of these results to mechanisms of cell death in Alzheimer’s disease will be discussed. Supported by the NZ Health Research Council.
673.17
S100P, a neurite extension factor, is overexpressed by activated astrocytes associated with amyloid containing plaques in Alzheimer's disease and has been implicated in dystrophic neurite formation in these plaques. We sought to correlate S100P-immunoreactive (S100P+) astrocytes with the formation of dystrophic neurites and thus with the linking of diffuse amyloid deposits into neuritic plaques. Double immunohistochemical labelling was used to determine the number of S100P+ astrocytes associated with different plaque types in temporal lobe of 12 Alzheimer patients, age 65±9 years. A total of 80 plaques were examined and the plaques were classified into four types according to the pattern of amyloid distribution (diffuse vs dense core) and the presence or absence of B-amyloid precursor protein (β-APP) immunoreactive dystrophic neurites. We found that non-neuritic plaques had small numbers of anomalous S100P+ astrocytes (1.3±0.1 cells/plaque; mean ± SEM; 80% of plaques had associated S100P+ astrocytes). In contrast, diffuse neuritic plaques had the most associated S100P+ astrocytes (4.2±0.2, 100%). Dense-core neuritic plaques had fewer S100P+ astrocytes (1.6±0.2; 90%). Dense core non-neuritic plaques were almost devoid of S100P+ astrocytes (0.15±0.05; 12%). Computerized image analysis showed that the number of plaque-associated S100P+ astrocytes significantly correlated with the cross-sectional area of plaque β-APP immunoreactivity, an index of the size of the plaques' dystrophic neurite shells (R = 0.66; P < 0.05). These results are consistent with a role for S100P in the induction and maintenance of dystrophic neurites in plaques of Alzheimer's disease. Supported in part by NIH AG02038, NS27414, and AG12411.

ALZHEIMER'S DISEASE: NEURONAL INJURY AND DEATH

Although cognitive impairment in aging and Alzheimer's disease (AD) is mainly associated with neuronal alterations, the cerebrovascular system also undergoes prominent changes. With electronmicroscopic (EM) techniques we previously showed progressive decline of the microvascular wall in the cerebral cortex of aged rats. Aged rats show basement membrane thickening, massive perivascular collagen deposits and pericyte degeneration, which suggests a hampered transport function of the blood brain barrier.
The importance of the cerebral microvascular condition and blood supply for cognition was studied (1) in AD by EM analysis of the microvascular wall and (2) by estimating the cerebral blood flow in AD patients. The cerebrovascular system also underwent similar changes as identified in the EM. These findings suggest that changes in microvascular condition and blood supply for cognition are present in AD as well.

Diabetes mellitus and Alzheimer's disease (AD) are significant health care problems in the elderly population. The present study examines the co-occurrence of diabetes and AD in a large, well-characterized patient population, and examines the contribution of vascular disease history to diabetes prevalence among dementia groups.
The assessment records of 275 dementia patients were evaluated by diagnostic group for the existence of diabetes and vascular disease risk factors (hypertension, heart disease, stroke, hyperlipidemia, etc.). Diabetes occurred significantly less often in AD patients than in patients with other dementias. Vascular disease, which is closely related to epilepsy, was more prevalent in AD patients than would be expected given the exceptionally low occurrence of diabetes in this group. Further, there were no differences among dementia groups in fasting glucose levels.

We reported decreased levels of gene expression and enzyme activity of cytochrome oxidase, a mitochondrial enzyme of oxidative phosphorylation, in affected brain areas in Alzheimer's disease. In this report, we used several lines of evidence to examine the hypothesis that energy impairment in neurons of AD cause neurodegeneration observed in AD brain. We extended our observations by analyzing the level of gene expression of a subunit of another mitochondrial enzyme, NADH dehydrogenase (complex I of oxidative phosphorylation) in this report. First, we raised monoclonal antibodies (mAbs) against a conserved cytosolic core sequence of a normal human brain by applying a modified hybridomas technique (SOFASTIC immunization). One mAb, BIOS, immunohistochemically detected an antigen only in the mitochondrial subcellular fraction separated from other organelles in the rat brain. We further isolated a cDNA clone immunoreactive to mAb BIOS by screening an expression cDNA library and found that the cDNA fragment is a portion of ND4 subunit of NADH dehydrogenase.
We characterized the localization and the level of expression of ND4 in adult brains and cultured cells of rat and human and immunohistochemical and molecular biological procedures. Finally we compared the level of ND4 gene expression in normal human brains to that in AD brains. We found that ND4 is highly expressed in the cytoplasm of large neurons and that immunohistochemical staining of brain sections showed a significant decrease of ND4 in several brain areas in AD, without a significant decrease in the number of neurons.
Northern blot analysis also showed a significant decrease of ND4 in AD. These results indicate that gene expression of mitochondrial DNA-encoded enzymes is generally decreased in AD and suggest that the energy impairment in selective neurons may be involved in neurodegeneration in AD.
674.5 MITOCHONDRIAL MEMBRANE FLUIDITY AND OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IN AGED AND AD HUMAN BRAIN. L. M. Mocnay, T. R. Cocchi, M. C. Pelcic, L. A. Cherubini, E. Chioléro, L. Altschuler, G. Romani, U. Sensi. 1Department of Clinical Medicine, Pathology and Pharmacology, Perugia University, Perugia (Italy); 2National Lab. Neuroimmunology, Massachusetts General Hospital Harvard Medical School, Boston (USA); 3Department of Medical Physics, Politechnical Institute, Perugia (Italy); 4Department of Biochemistry and Medical Chemistry, Perugia University, Perugia (Italy).

Oxidative damage on biological molecules has been proposed as a major cause of alterations observed in aging brain as well as in neurodegenerative diseases. In this study we measured membrane fluidity in mitochondria extracted from three cerebral regions and cerebellum of Alzheimer's disease (AD) patients and age-matched controls by means of fluorescence polarization technique. A significant reduction of mitochondrial membrane fluidity was found in AD, except in cerebellum. In controls, a decrease of membrane fluidity was observed along with age and it was also related to the content of the oxidized nucleoside 8-hydroxy-2'-deoxyguanosine (OHdG) in mitochondrial DNA (mtDNA). Alterations in membrane fluidity seem to be mainly a result of lipid peroxidation since it dramatically decreased when mitochondria were exposed to FeC3 and H2O2. The parallel increase of viscosity in mitochondrial membrane and amount of OHdG in mtDNA is suggestive of a relationship between these biological markers of oxidative stress, supporting the hypothesis of a membrane mediated excretion of DNA. From these results in AD patients biological molecules seem to be particularly sensitive to oxidative stress suggesting that free radicals, together with aging, may play a fundamental role in the pathogenesis of the disease.


One of the early symptoms of Alzheimer's disease (AD) is characteristic deposition of 8-amyloid peptide (Aβ) among brain regions. Although prevention of brain amylogenesis is thought to be the plausible approach to halt or slow down the progression of AD, the lack of ability to understand the progress of the research. We, however, previously reported close temporal and spatial relationships between deposited structures of PEP and Aβ in SAM hippocampus, suggesting that SAM could be used as an in vivo model for testing therapeutic compounds. The purpose of this study was to estimate whether chronic treatment of SAM mice with a non-peptide PEP inhibitor, Y-2974, could suppress the progression of amylogenesis in the hippocampus. Male SAM (12-month-old age) were given oral doses of Y-2974 (1, 10, and 20 mg/kg/day) in drinking water from 2.5 to 8 month-old Paraffin-embedded coronal sections of the hippocampus were made to stain Aβ with polyclonal anti-Aβ (1-12). Aβ-like immunoreactivity (Aβ LI) was analyzed by computerized image analyzer and NIH Image.

Deposited structures of Aβ-LI which were composed of fine granules were observed in the hippocampus of SAM. After the repeated dosing of Y-2974, the number of granules per deposit, and total number of Aβ granules were decreased. Moreover, Y-2974 decreased density of characteristic lysosomal distribution of the mean density of the granules. These results indicate that Y-2974 suppresses the amylogenesis in the hippocampus of SAM, providing further support of our working hypothesis that PEP is closely involved in the amylogenesis, and that Y-2974, an inhibitor of PEP, would be promising as a therapeutic agent for AD.

674.9 BCL-2 PROTEIN IMMUNOREACTIVITY INCREASES IN ALZHEIMER'S DISEASE BRAIN WITH DISEASE SEVERITY. Takao Satoh, Brian J. Cummings and Carl W. Combs*, Institute for Brain Aging and Alzheimer's Disease, University of California, Irvine, Irvine, CA 92717-4500.

Bcl-2 has been suggested to be one of the proteins preventing apoptosis in a variety of cell types. Recently, apoptosis has been suggested to have an important role in the pathogenesis of Alzheimer's disease (AD). We utilized Bcl-2 immunohistochemical methods to examine Bcl-2 in the hippocampus and entorhinal cortex of AD patients ranging in clinical and pathological severity from mild to severe and compared these results to those from controls. Immunoreactivity for Bcl-2 was increased relative to controls in most neurons of the entorhinal cortex, subiculum, C1, C2, C3, hilus and dentate gyrus granule cells. Bcl-2 staining increased with increasing disease severity. However, neurons displaying increased immunoreactivity for markers of neurotrophic factor formation and neurite formation showed reduced staining for Bcl-2, suggesting that Bcl-2 may be downregulated in these neurons. Immunoactivity for Bcl-2 and the vasculature was also increased in AD. These results suggest that Bcl-2 protein may have a role in compensation responses to AD pathology, perhaps allowing for the remaining neurons a margin of protection from apoptosis.

674.10 PATTERNS OF NEURONAL DNA FRAGMENTATION DETECTED BY TUNEL IN ALZHEIMER'S DISEASE. R. Halburt**, J. T. Troncoso**, C. Kastin**, Y. K. Kalopoulous. 1Neuropathology Laboratory, Dept. of Pathology, Neuroscience, and Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Alzheimer's disease (AD), the most common type of dementia, is characterized by cerebral atrophy, 8-amyloid protein (Aβ) deposits/senile plaques, neurofibrillary tangles, and loss of synapses. Although morphometric studies of different areas of the CNS demonstrated loss of pyramidal neurons in neocortical layers III and V in AD, the precise extent and distribution of cortical neuronal loss remains undetermined. In the present study, we used terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) as a method for detection of fragmented DNA to identify degenerating neurons in the cerebral cortex of individuals with Alzheimer's disease. In late stages of AD, TUNEL-labeled neurons were found in all layers of the above cortices as well as in the hippocampus. The specificity of TUNEL labeling was supported by the absence of labeling in controls. In conclusion, our findings of the laminar pattern of neuronal degeneration suggest that specific neural systems are involved in the pathogenesis of AD and that the TUNEL method can be used as a method for direct demonstration of neuronal DNA fragmentation in early stages of disease.
674.11

Neuronal death is a prominent feature of Alzheimer’s disease (AD) and has been suggested to play a significant role in the expression of dementia in AD and perhaps other age-related cognitive function. Recent studies in vitro have suggested that apoptosis may play a role in the neuronal cell death associated with AD. We have recently demonstrated the presence and morphological features of apoptotic-like nuclei in AD brain and have shown that such apoptotic nuclei are present in a subset of neurofibrillary tangles in AD brains (Neuroreport 5, 2529-2533, 1994). In this study, we examined the hippocampal formation of AD brains using a TdT-end labeling system (ApopTag, Oncor) on vibratome sections to quantitatively assess apoptotic-like nuclei. The vast majority of apoptotic-like nuclei were located in the grey matter, and were found within neurons. 2/3 of PHF-tau positive neurons were associated with apoptotic-like nuclei. Apoptotic-like nuclei were generally not detected within extracellular neurofibrillary tangles. These results suggest that neuronal cell death may occur without neurofibrillary formation, and neurofibrillary tangle bearing neurons may die by apoptosis.

In conclusion, our data indicate that neuronal DNA damage appears to be more prominent and widespread in AD than previously realized. This could reflect either a mechanism of cell death, DNA damage/repair mechanisms, or both. In this context, TdT labeling may be an early marker of cellular risk.

674.13

This experiment was designed to investigate the duration of bioactivity of intracerebrally grafted transgenic NNGF producing fibroblasts by transplanting the fibroblasts at various time-points before fimbria-foremice (FmFm) lesioning. Syngeneic Fischer dermal fibroblasts were grown in culture (<8 passages) and transduced with a retroviral vector encoding the NNGF, cDNA or E Cell lac-z both driven by the internal viralLTR promoter in vivo. NNGF production determined by an ELISA assay (257 ng/10^6 cells/24 hr) was comparable to that reported previously (Rosenberg et al., Science 242:1575-1578, 1988). Each rat received 2 grafts of 1 ml of 7.5 X 10^6 of NNGF (w+/+) or lac-z producing cells (w-/-) in the medial septum (MS). Immediately following the implantation surgery (day 0) or following variable delay intervals (4, 7, 14, 28 & 42 days), a unilateral FP lesion was performed ipsilateral to the cell implantation. The rats were sacrificed 21 days after the FP lesion. Fixed tissue was sliced at 40 mm and prepared for choline acetyl transferase (ChAT in MS) immunohistochemistry, acetylcholinesterase (AChE) histochemistry (hippocampus), and hematoxylin and eosin staining. Some animals were removed from the study prior to ChAT+ cell counting by evaluating their FP lesion via examination of the ACAn stained sections of the hippocampus. Each MS section was evaluated "blind" to treatment by counting ChAT+ cells and by noting whether the graft was innervated by ChAT+ fibers. There was no effect of surgery or on ChAT+ cell survival in the lac-z group, therefore the lac-z data were combined into one control group for further analysis. NNGF-producing fibroblasts significantly enhanced survival of ChAT+ neurons at all FP lesion delays (p < 0.05) but the magnitude of the survival effect appeared to wane over time (42 day percent survival < 14 day survival, p < 0.05).

675.1

Pretreatment with brief (2 min) ischemia induced tolerance for subsequent ischemia (5 min) and prevented delayed neuronal death (DND). Using monoclonal antibody against bcl-2 oncogene we previously reported that immunoreactivity of bcl-2 greatly increased in the gerbil hippocampal CA1 sector in parallel with acquisition of tolerance for DND (Shimazaki et al. Neurosci. Res. 20:95,1994). To determine time course and locus of bcl-2 production after sublethal brief ischemia, we have studied mRNA expression in the gerbil hippocampus by in situ hybridization. The level of bcl-2 mRNA was markedly increased at 2 days following 2 min ischemia. Northern blot analysis supported the result showing 2-fold increase in bcl-2 mRNA after 2 min ischemia. In the gerbil treated with 5 min ischemia, no increase in the level of bcl-2 mRNA was observed. These results suggest close correlation between acquisition of tolerance for DND and the expression of bcl-2, which is known to support survival of the neuronal death.

675.2
TRANSIENT FOCAL ISCHEMIA ELEVATES bcl-2 EXPRESSION IN THE NEOCORTEX OF ZO HG,*, A.M. Bhutan* and D.R. Robertson†. National Research Council of Canada, and Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Canada.

Several lines of evidence suggest that apoptotic mechanisms may contribute to neuronal death following transient cerebral ischemia. Over-expression of the proto-oncogene bcl-2 in several cell lines has recently been shown to reduce apoptotic death produced by a variety of noxious treatments. This finding suggests that there may be a positive relationship between bcl-2 expression and resistance to ischemic cell death in vivo. The present study addressed this hypothesis by examining the effects of transient forebrain ischemia on bcl-2 expression in the neocortex. Reversible focal cerebral ischemia was produced in male spontaneously hypertensive rats by 90 min of transient right middle cerebral artery occlusion (MCA-o) combined with permanent right common carotid artery occlusion. Six groups, composed of 4 animals each, were subjected to 90 min of MCA-o and sacrificed after 1, 4, 8, 12, 24 or 48 hr of reperfusion (RP). A seventh group served as sham-operated controls and underwent the same operative procedures except that the MCA was not occluded. Brains were rapidly removed, quickly frozen and sections 12 µm thick cut through the cortex. bcl-2 mRNA was detected by in situ hybridization histochemistry and quantitated using an image analysis system. bcl-2 mRNA levels were low in the neocortex of sham animals. Transient MCA-o elevated bcl-2 mRNA levels selectively in the ischemic penumbral region of the neocortex within 8-12 hr with peak increases occurring between 12-24 hr of RP. The results suggest that elevated bcl-2 expression may contribute to the survival of neurons located in the penumbral region following cerebral ischemia.
ISCHEMIA: ISCHEMIC TOLERANCE AND STRESS PROTEINS

675.3
There is increasing evidence for the involvement of apoptosis in the selective, delayed neuronal death resulting from cerebral ischemia. Bcl-2 is present in the developing and adult neural system and has been shown to prevent apoptosis and neuronal cell death under many neurotoxic conditions implicated in ischemia. To investigate the possible relationship between bcl-2 and selective neuronal death, we examined the distribution of bcl-2 in the rat hippocampus following transient forebrain ischemia (TFI). TFI was induced in normothermic adult rats by bilateral carotid artery occlusion with hypothermia. Animals were perfused with 4% paraformaldehyde 6, 12, 18, 24, 48, 72 hours following induction of TFI. Frozen 10 μm coronal sections were analyzed for the distribution of bcl-2 protein by immunohistochemistry. Sections were immunostained using a polyclonal rabbit antibody specific for bcl-2 (Santa Cruz) or antibody preincubated with the immunizing peptide. Following incubation with biotinylated anti-rabbit IgG, sections were incubated with avidin-biotin-peroxidase complex and DAB was used as a chromagen.

675.4
Recent data suggest that Bcl-2 expression provides neuroprotection in models of cerebral ischemia. In the current study we show that Bcl-2 acts as an antioxidant and may affect patterns of Ca^2+ metabolism, the exact mechanism is unknown. The effect of Bcl-2 expression in GT1-7 neural cells on mitochondrial resistance to damage induced by high concentrations of extramitochondrial Ca^2+ has been examined. Bcl-2 appears to have little effect on state 3, state 4, or uncoupler-stimulated rates of respiration of digitonin-permeabilized cells under control conditions. However, the addition of 7.7 μm of CaCl_2 to cells incubated uncoupler-stimulated respiration of permeabilized control cells by approx. 83%, whereas this rate in Bcl-2 expressing cells was inhibited by only 30%. Measurements of maximal Ca^2+ uptake capacity prior to Ca^2+ release revealed that control mitochondria could sequester 600 and 500 nmoles Ca^2+ for 4.3 and 10 minutes, respectively, whereas Bcl-2 expressing cells could accumulate 1013 and 800 nmoles under the same conditions. In addition, mitochondria isolated from Bcl-2 expressing cells were significantly less sensitive to respiratory inhibition in response to 42-167 nmoles Ca^2+ mg mitochondrial protein. The ability of mitochondria of Bcl-2 expressing cells to maintain function under conditions of cytoxic levels of extramitochondrial Ca^2+ may provide a mechanism by which Bcl-2 inhibits delayed neuronal death following cerebral ischemia. (Supported by NIH NS34132)

675.5
REGULATION OF GENE EXPRESSION BY O2 DEPRIVATION IN DROSOPHILA MELANOGASTER. Embo Ma and G. G. Haddad*, Departments of Pharmacology and Cellular Physiology, Yale University School of Medicine, New Haven, CT 06520
We have recently shown that the adult fruit fly Drosophila melanogaster is only tolerant to complete deprivation of O2 and can totally recover from 4 hr of anoxia. To study the mechanisms that are activated during O2 deprivation and which are potentially protective, we used two groups of DM: one was kept in 0% O2 for 4 hr and another was kept as a control. Flies from both groups were immediately frozen in liquid N2, shaken vigorously, and passed through a number of sieves of successively smaller pore sizes in order to collect poly-A RNA. RNA isolated from both groups of DM heads and the transcripts for heat shock proteins (HSP70 and HSP62), ubiquitin (UB3 and UB4), cytochrome oxidase (COX) and superoxide dismutase (SOD) were analyzed by slot blot and Northern blot analysis. Gene expression for HSP70 was up-regulated by ~3.5 times while UB4 and COX were down-regulated to about the same extent as noted above. SOD, however, was not affected. From these results we conclude that during O2 deprivation there is differential regulation of gene expression and 2) the up-regulation of HSP70 and down-regulation of UB4 are consistent with the idea that such changes potentially protect against protein degradation. (Supported by NIH T32HL07777, PO1 HD32573, HL28840 and NS52578).

675.6
AN OPTIMIZED MODEL OF INDUCED ISCHEMIC TOLERANCE IN THE GERbil. H. Abe and T.S. Nosaka, Jr*, Dep. of Neurology, University of Tennessee, Memphis, TN 38163
Brief insults moderate the damage seen following subsequent more severe ischemic challenges, but reported effects are highly variable. In the present study we have investigated hippocampal DC potentials to determine effective durations of priming insults and test challenges, identifying threshold depolarization intervals for tolerance induction and changes in gene expression. Ischemia of varied duration was produced by bilateral carotid artery occlusion in halothane-anesthetized gerbils (n=25) fixed in a stereotaxic frame. Hippocampal DC potentials were recorded via glass microelectrodes. Induced mRNAs were evaluated in situ hybridization at 1 hr or 6 hr reisultation. In other gerbils a test insult was administered 2 d after the first and consisted of depolarization for 2385 sec, which produced maximal neuronal loss in naive animals. Histological evaluation of CA1 was done 7 d after the test insult. Tolerance was first evident after 290 sec depolarization, with complete protection after 2150 sec. The mRNA encoding the stress protein, hsp72, was not increased after depolarizations ≤120 sec, with consistent induction only after ≥200 sec, and was strongly expressed only after ≥240 sec, which was the threshold for histological damage. In contrast, slight increases in c-fos, junB and junD were detected after the shortest depolarizations (60 sec) and these mRNAs were reliably induced in all hippocampi after ≥250 sec. These results: 1) indicate that the stress response is not required for induced ischemic tolerance, 2) demonstrate that diverse cascades of altered gene expression must occur after insults that induce tolerance, and 3) establish a highly reproducible model in which to study neuroprotective mechanisms after repeated insults.

675.7
In gerbil model of unilateral middle cerebral artery occlusion (MCAO), in which both sides of hippocampus are spared from ischemic insult, hsp70 and c-fos mRNA induction in the hippocampus was studied using in situ hybridization method. Ischemic tolerance of the hippocampus was also investigated in a second transient forebrain ischemia at 3-7 days after MCAO. Induction of hsp70 mRNA was faint in both hippocampal regions at 12 to 24 hr after left MCAO, while c-fos mRNA was not detected in the left hippocampus at 0.5 to 6 hr following ischemia. Animals undergoing left MCAO displayed ischemic tolerance at 9 min bilateral carotid artery occlusion (5000 sec) after MCAO. More than 40% of CA1 neurons survived in the left hippocampus. In contrast, less than 10% of those survived in the right. No such tolerance occurred at 7 days after MCAO. The dissociation of hsp70 and c-fos mRNA induction after MCAO may be derived from the difference of threshold between these two mRNAs to play little role in the induction of focal ischemic tolerance in the gerbil MCAO model.

675.8
The objective of the present study was to determine whether cortical spreading depression (CSD) induces tolerance to ischemia in the cerebral cortex of rats. CSD was evoked by applying 2 M KCl to the intact dura over the frontal cortex of one hemisphere for 2 hr. After recovery for 24 hr, bilateral forebrain ischemia was produced using carotid artery occlusion and arterial hypotension. Following ischemia for 6 min (n=7) or 10 min (n=6), animals were permitted to survive for 4-6 days prior to assessment of histopathology. In separate experiments (n=5), CSD evoked 11 ± 3 DC shifts and triggered the expression of c-fos mRNA, but not hsp72 mRNA, in the ipsilateral cortex. Forebrain ischemia of 6-min duration caused severe excitotoxic neuronal necrosis in many brain areas, but the number of necrotic neurons in the cerebral cortex preconditioned with CSD was significantly lower than that in the contralateral cortex. The extent of neuronal necrosis in striatum and hippocampus was not significantly different between hemispheres. Forebrain ischemia of 10-min duration caused a severe degree of neuronal necrosis in the cerebral cortex, but the extent of neuronal necrosis induced by CSD was markedly diminished relative to that in the contralateral cortex. These results demonstrate that CSD induced severe ischemia in cerebral cortex by mechanisms unrelated to hsp72.
675.9

THE EXPRESSION OF BFGF INCREASED AT TRANSCRIPTION LEVEL BUT NOT AT TRANSLATION LEVEL IN THE CA1 PYRAMIDAL CELL LAYER IN ISCHEMIA-TOLEANCE INDUCED RAT MODEL.

M. Indoh, T.A. Nagoya, Y. Yokogawa, S. Nagaoka, K. Kimura

Dep.of Neurosurgery, Hamamatsu University School of Medicine, Hamamatsu, Japan

We previously reported that 10 minutes of transient forebrain ischemia induces BFGF mRNA but not BFGF protein in the CA1 pyramidal cells. In this study, we investigated the changes in following 24 hours of transient forebrain ischemia, that is so called ischemia-tolerance induced rat. Transient forebrain ischemia was imposed with 4 vessel occlusion method. In situ hybridization demonstrated that 2 minutes of transient forebrain ischemia induced BFGF mRNA in the CA1 pyramidal cell layer by 24 hours after ischemia. But immunohistochemistry failed to show the expression of BFGF in the CA1 pyramidal cell layer. Other investigators have recently reported that ischemia induced BFGF protein in neurons shortly after ischemia in vitro. However, our results do not suggest that BFGF plays a key role in the protection for neurons in ischemia-tolerance induced rat.

675.11

CHRONIC HYPOXIA INCREASES THE NEURONAL RESPONSE TO ACUTE HYPOXIC STRESS. J.P. O'Reilly and G.G. Haddad

Depos of Neurology, Psychiatry, Yale University, New Haven, CT.

Acute oxygen deprivation can have severe consequences in the central nervous system (CNS). However, much less is known about the effects of long-term (chronic) hypoxic exposure on the CNS. We hypothesized that exposure to chronic hypoxia would be detrimental to the CNS. We exposed rats (P0-P2) to FiO2 of 0.15 and 0.15 P50 (exposed). Membbrane potential (Vm) and input resistance (Rm) were measured during in vitro acute ischemia at two levels of tissue Po2 in neocortical neurons (NCX) and hippocampal neurons (HPC) using the brain slice technique and intracellular recordings. Age-matched normoxic animals were used as controls (naive). In response to acute hypoxia (15 Torr), exposed NCX (n=13) depolarized to a much greater extent (51.2±7.0 mV; p<0.001) and showed a lower decrease in Rm (57.6±2.8% vs naive NCX (4.8±10 mV, n=19; p<0.001). Exposed (n=25) and naive (n=33) XII showed similar responses to both hypoxia and anoxia. Re-oxygenation produced a rapid recovery of both Vm and Rm in naive XII and also in exposed XII. However, exposed NCX remained depolarized during the recovery period (100 min) following both hypoxia and anoxia, and Rm showed a delayed recovery pattern. In addition, 28% of the exposed NCX failed to recover from anoxia. We conclude that chronic hypoxic exposure renders neurons in the NCX more sensitive to the effects of acute hypoxia, and possibly more vulnerable to stress-induced injury. (Supported by HD 32573, HL 28940, NS 32578)

675.13

"ISCHEMIC TOLERANCE" IN CORTICAL CELL CULTURE. M.C. Grabbe, and D.W. Cho

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Several studies in recent years have established the phenomenon of "ischemic tolerance" in animal models of brain ischemia, where mild ischemic insults render the brain resistant to injury induced by a subsequent, more severe insult (Kissaga, Br. Res. 528:21, 1990; Kimura, J. Cereb. Blood Flow Metab. 11:299, 1991). This phenomenon is thought to reflect protective cellular alterations induced by the first, preconditioning insult that are maintained after performance to test if the phenomenon could be demonstrated in cell culture.

Murine cortical cell cultures containing both neurons and glia (DIV 13) were exposed to a mild 60 min ischemia (2.5-20 min) to brief to induce detectable cell death. 24 hrs later, these cultures were exposed to a 50-75 min period of oxygen-glucose deprivation sufficient to induce intermediate levels of neuronal death without global death in controls. The present death was not much lower than those preceded by sham wash alone. These experiments suggest the idea that mild ischemic insults can render cortical cells resistant to subsequent ischemic insults, and provide encouragement for further examination of this phenomenon in the in vivo setting. Supported by NIH NINDS grant NS 30337 (DWC).

675.10


Previous studies have shown that prior exposure to brief periods (e.g. 1.5-2 min) of ischemia protects against subsequent, ischemic insult several days later. It is not known if this ischemic "tolerance" translates into functional preservation since a number of treatments maintain neuronal viability but not function.

Gerbils were exposed to 2 x 1.5 min episodes of global ischemia (24 hr apart) followed 3 days later by a 5 min occlusion that typically destroys >95% of CA1 neurons. In one experiment, animals were tested in an open field test of habituation 3, 7 and 10 days after the last ischemia. In a second experiment CA1 field potentials (TEPS) were recorded from hippocampal slices taken 4 days after the last ischemia. CA1 cell counts showed that preconditioning resulted in about 70% preservation assessed 10 days after ischemia. In spite of the substantial degree of neuroprotection, open field activity levels in preconditioned gerbils were no different than ischemic gerbils, indicating a marked habituation deficit. Similarly, CA1 TEPS were less than 50% of those recorded in sham animals. These data suggest that while ischemic preconditioning protects CA1 neurons histologically, many of these neurons are functionally abnormal.

Supported by the MRC of Canada.

675.12

ALTERNED NEURONAL PROPERTIES IN NEOCORTEX FOLLOWING LONG-TERM EXPOSURE TO HYPOXIA. M.L. Schwab*, J.P. O'Reilly and G.G. Haddad. Dept. of Neurobiology, Biology, Pediatrics, and Cellular and Molecular Physiology, Yale University and School of Medicine. New Haven, CT, 06520.

Neurons in the central nervous system (CNS) are extremely sensitive to oxygen availability in the environment, and oxygen deprivation can have severe physiologic and pathophysiologic consequences. Since little is known about the effects of long term oxygen deprivation in the developing CNS, we studied neurons from animals chronically exposed to a hypoxic environment. We hypothesized that chronic hypoxic stress would be detrimental to the development of the CNS. Neonatal rats (P0-P2) were raised in a hypoxic chamber with FiO2 of 9.5 ± (0.1). At P21-P35, the animals were removed from the chamber, and the neocortex (NCX) was prepared for morphologic and electrophysiologic studies. Golgi stained sections (100 μm) were used to count spine density on apical dendrites of pyramidal neurons in NCX. Data from 6 exposed and 6 matched normotic controls (naive) revealed a significant decrease (p<0.01) in the mean # of dendritic spines in the NCX from exposed (40±7 vs 17±3; SEM vs naive animals (49±13). Intracellular recordings from brain slice were used to measure membrane potential (Vm) and input resistance (Rm). Baseline Vm were not different between exposed and naive NCX, while Rm were greater (p<0.01) in exposed (53.5 vs 34.4 MΩ, n=40) compared to naive (42.8 ± 2.3 MΩ, n=52). We conclude that 1) long-term exposure to a reduced oxygen environment alters neuronal cytosarchitecture and membrane properties in NCX, and 2) these changes may play a role in the increased response to acute hypoxia in NCX neurons from exposed animals. (Supported by HD 32573, HL 28940, NS 32578)

675.14


Pre-exposure of rat hippocampus to short periods of hypoxia increases the resistance of CA1 pyramidal neurons to longer, normally fatal hypoxic insults. We have previously shown that changes in gene transcription result from oxygen deprivation and that these changes are accompanied by RNA binding proteins that associate with the hypoxia responsive element (HRE). We have identified a novel protein (HIF) that contains an oxygen-sensitive domain, and is a regulator of this heme-containing sensor to its deoxygenation conformation, triggering the expression of neuroprotective gene products. Carbon monoxide (CO) can replace O2 as the HIF inhibitor, making heme a potential target for gene therapy. We have demonstrated that CO inhibits the HIF-1alpha gene in an oxygen-dependent manner.

To determine if hypoxia and CO alter gene expression, we performed an analysis of hippocampal gene expression using a microarray of over 16,000 genes. Hypoxia significantly increased the expression of the heme oxygenase-1 (HO-1) gene. This gene is known to be induced by hypoxic conditions. We have also identified a CO-binding protein that regulates HO-1 expression. This protein is a member of the CUB (complement C1r/C1s, Hageman factor, and properdin) superfamily of proteins. We have identified a heme-containing sensor that is regulated by hypoxia. This sensor is a transcription factor that regulates HO-1 expression.

In conclusion, hypoxia and CO induce HO-1 expression in hippocampal cells. This is the first report of a heme-containing sensor that regulates HO-1 expression. This finding may have implications for the treatment of neurodegenerative diseases.
675.15


Ischemic brain injury leads to a destructive cascade of events that culminates in the death of many neurons in hippocampal, striatal and cortical areas. The mechanism by which this occurs is poorly understood, but one of the early events following injury is a rapid increase in the expression of immediate-early genes and stress genes such as the inducible Hsp-70. In order to determine the progression of these changes initiated by ischemic insult that eventually lead to cell death, we used young rats in the Rice-Vannucci model of ischemia-hypoxia and have studied the effects of 60 min hypoxia) injury on the induction of c-fos, c-jun and hsp-70 in the hippocampus. Rats (100) were divided into 4 groups (naive control, hypoxic control, ligated control and ligated hypoxic). Two animals that had seizures were taken out of the study. No cell death or increased protein expression was seen in any of the control groups. However, in the ligated hypoxic group, severe damage was observed. Such severe injury was associated with increased expression of the c-Fos and c-jun proteins on the ipsilateral side to the injury at 12 hrs. However, at 24 hrs the expression of both c-Fos and c-jun proteins on the ipsilateral side had returned to basal levels while their expression was increased on the contralateral side. Hsp-70 levels increased on the ipsilateral side in CA1 at 24 hrs, but not at 12 hrs. At 48 hrs, the levels of c-Fos and c-jun are at basal levels, but Hsp-70 remains elevated on the ipsilateral side. At no time was Hsp-70 protein expressed on the side contralateral to the insult. These findings suggest that even in this severe type of ischemic insult, expression of c-fos, c-jun and hsp-70 may be involved in the events leading to cell death. Altering expression of these genes may therefore be expected to change the degree of injury. (Supported by the MRC and SmithKline Beechem Pharma Inc.)

675.16

NEURONAL AND GLIAL EXPRESSION OF INDOUCIBLE HSP70 IN FOREBRAIN ISCHEMIA. D. Xie1, J.-C. Plummer2, H.A. Robertson1 and R.W. Currie1. Laboratory of Molecular Neurobiology, Deps. of Anatomy & Neurobiology and Pharmacology2, Dalhousie Univ., Halifax, NS, Canada, B3H 4J7.

Although induction of HSP70 in the vulnerable regions in the brain after cerebral ischemia has been well documented, the relationship of this response with neuronal death and survival remains unclear. In the present study, we examined neurons as well as glial cells for cell death using cresyl violet staining, and for expression of HSP70 and c-Fos using immunohistochemistry at 1.5, 6 and 120 hours after 30 minutes of global ischemia. Immunoreactivity against HSP70 was first observed at 12 hours in neurons in layer V of the cortex, and at 24 hours in layers II and III. Localization of the HSP70 protein was seen in neuronal bodies and the nucleus of HSP70. Most of the pyramidal cells in the hippocampus expressed HSP70 at 24 hours and the expression remained for high levels until 120 hours. Elevated expression of HSP70 remained until 120 hours in the CA1 region. This pattern of HSP70 expression was seen when there was only moderate cell loss in CA1 at 72 and 120 hours as revealed by cresyl violet staining. In some cases, cresyl violet staining revealed abrupt and massive neuronal loss due to cell death in CA1 at 24 hours and in CA3 and dentate gyrus at 72 hours. In these brains, at 24 hours, HSP70 was not observed in CA1 but CA1 had a pattern similar to brains with only moderate cell loss in CA1. Only in cases with severe cell loss, was HSP70 expression seen in layer VI of the cortex. HSP70 expression was observed in glia-like cells, often in clusters, in areas in cortex and hippocampus where neuronal HSP70 immunoreactivity was no longer detected and cresyl violet staining revealed no loss of neurons. These glial-like cells were immunoreactive for OX-42. These findings suggest a relationship between the timing of HSP70 expression and survival. Glial expression of HSP70 may be associated with activation of the glial cells in response to neuronal necrosis. (Supported by the MRC of Canada and SmithKline Beechem Pharma Inc.)

675.17

EXPRESSION OF hsp70 mRNA FOLLOWING TRANIENT BILATERAL COMMON CAROTID ARTERY OCCLUSION IN MICE. ISCHEMIA-MEDIATED OVEREXPRESSION of CuZn-SUPEROXIDE DISMUTASE. M. Honkaniemi1, K. Konttinen1, J. Mikawa2, S. Chan3, C. Sharp4, C. Ellis5, and Chan P.P.6. (1) Department of Neurology, (2) Neurosurgery and (3) Pediatrics, University of California, San Francisco, CA 94143 (4) Department of Neurology, VA Medical Center, San Francisco, CA 94121.)

Various studies have demonstrated an increase in heat shock protein 70 (HSP70) synthesis in the brain following transient ischemia, suggesting a protective role for HSP70 against ischemic insult. Recently, it has been reported that HSP70 mRNA (hsp70) expression is affected by oxidative stress. However, the underlying mechanism is unclear. We used transgenic (Tg) mice which overexpress CuZn superoxide dismutase, and measured the amounts and localization of hsp70 expression following transient bilateral common carotid artery (CCA) occlusion. Male heterozygous Tg mice (Tg/C57BL-125) and nontransgenic (WT) normal littermates (1548 g) were anesthetized with ketamine/xylazine. Bilateral CCA occlusion was produced for 3 min using small surgical clamps. The mice were decapitated, and brains were frozen and sectioned 20 μm thick. The sections at 24, 72, and 168 hrs after the surgery were processed for in situ hybridization with a 35S-labeled oligonucleotide probe of hsp70 (34). Histological findings were examined by cresyl violet staining, and DNA fragmentation was examined by terminal deoxynucleotidyl transferase (TUNEL) technique. In Tg mice, hsp70 was strongly expressed in cortex, caudate putamen, thalamus and hippocampus at 4 hrs, and it was still detectable within the hippocampus CA1 for up to 24 hrs. However, in WT mice, hsp70 weakly appeared only CA1 from 4 to 24 hrs. There was no observable neuronal necrosis in either Tg or WT by cresyl violet, and DNA fragmentation was not observed in either Tg or WT. The present study demonstrates that increased CuZn-SOD activity induced or permitted the hsp70 expression after transient bilateral CCA occlusion without any observable cerebral injury. We speculate that upregulation of hsp70 expression may be associated with decreased production of reactive oxygen species in CuZn-SOD Tg mice after a transient cerebral ischemia. (Supported by NS-15453, NS-53772, AG-08593.)

675.18

ASTROCYTE SURVIVAL AND HSP70 HEAT SHOCK PROTEIN INDUCTION FOLLOWING ACIDOSIS AND HEAT SHOCK. P. Narasimhan1, R.A. Swanson, S. Sagar, F.R. Sharp. Department of Neurology, University of California at San Francisco and SPVMC, San Francisco, CA 94124.

Though severe acidosis is an important mediator of brain infarction, recent evidence suggests that mild acidosis may also protect against ischemic injury. This HSP70 protective effect is induced by acidosis in cultured cells and in ischemic brain, and is known to protect cells against many types of injury. Therefore, we have determined whether induction of heat shock proteins protects cultured astrocytes against acidosis. Brief exposure of cultured cortical astrocytes to acid (pH 6.5 for 40 minutes) led to induction of hsp70 mRNA and HSP70 protein in these cells. Heat shock of the cultured cortical astrocytes completely protected the astrocytes from heat tolerant (45°C for 4 hours). In contrast, heat shock pretreatment and acid pretreatment sensitized the astrocytes to injury from acidosis 24 hour later. Hsp70 induced by pretreatment protected astrocytes against exposure to acid 48 hours later. These results suggest that induction of other stress proteins and acid shock proteins partially protect the astrocytes against damage produced by high concentrations of hydrogen ions.

675.19

ANOXIA/AGLYCHEMA INDUCES mRNA ENCODING THE 70 KDA STRESS PROTEIN, HSP72, IN RAT HIPPOCAMPAL SLICES. O. Zhou* and T.S. Nowak, Jr. Deps. of Anatomy & Neurobiology and Neurology, University of Tennessee, Memphis, TN 38163

In previous studies we examined changes in the expression of mRNAs and proteins that occur during hippocampal slice survival. Under optimal conditions hsp72 was not detected, MAP2 immunoreactivity was comparable to that of normal brain, and Fos and Jun staining were only moderately increased. We have now developed conditions that meet these criteria to develop an in vitro model that replicates many features of postischemic hsp2 expression observed in vivo. Vivobrations slices (400 mm) were prepared from hypothalamic slices and incubated at 34°C in standard artificial cerebrospinal fluid (ACSF) purged with 95% O2, 5% CO2 including 5 mM glucose and 1.5 mM Ca. The anoxic/aglycemic insult was produced by transfer to ACSF lacking glucose, replacing O2 with N2 and reducing Ca to 0.2 mM. After insults of 2-6 min slices were returned to normal ACSF for various intervals up to 4 hours. Slices were fixed in 4% paraformaldehyde and sectioned for in situ hybridization, immunocytochemistry and histology. Insults of 2-3 min duration resulted in minimal changes in normal MAP2 immunoreactivity during the time intervals examined. However, while anoxia/aglycemia of 4 min or longer produced rapid loss of MAP2 staining and histologically shrunken neurons. Hsp72 mRNA was detected after reoxygenation intervals of 1 hr or longer, with preferential induction in dentate granule cells and CA3 neurons. Preliminary results indicate maximal expression after 3 min in vitro ischemia. These findings demonstrate the potential utility of such preparations for studies of changes in gene expression after ischemia.
676.1 PREVALENCE OF TRAUMATIC BRAIN INJURY IN RUGBY AND LACROSSE PLAYERS. J. Liotta, P. Donovich*. Environmentally Neurotoxicology Lab., Dept. of Psychology, State Univ. of N.Y. at Binghamton, 13902-6000.

The present study of 59 athletes was done to see the amount of traumatic brain injury (TBI) that they have sustained while playing in their respective sports. An inventory of their sports history was also examined. The athletes currently play women's lacrosse or men's and women's rugby. The testing instrument was the Binghamton Head Injury Questionnaire. Two trends were identified. The first being that rugby players had a higher incidence of TBI than lacrosse players. The second was that female rugby players had a higher incidence of TBI on avg, than males in both their sports history and in rugby. It was concluded that this may be due to the amount of experience that both sexes have with contact sports. Females may have reported every incident while males only reported the severe cases because they are used to big little blow. Another conclusion was that males have more developed necks and their heads are more protected as a result.

676.3 EFFECTS OF SECONDARY INSULT ON TRAUMATIC BRAIN INJURY. D.A. Chorney, J.S. Soboloff, L.L. Colgin, J.P. Davidson, and J.H. Green*. Dept. of Neurosurgery, LSU Medical Center, New Orleans, LA 70112

Secondary insults (e.g. hypotension, hypoxia) potentiate traumatic brain injury. We quantified the effects of traumatic brain injury followed by 10 minutes of hypotension (MAP 50 mm Hg) on rodent sensory/motor behavior. Brain-injured rats were injected in the right sensory/motor cortex by a piston with a 4x11nm elliptical tip which depressed the dura lama. Impact speed was equated by removing 3ml of blood via a jugular cannula. We reinfused the blood after the 10-min hypotensive period. Animals were placed in one of four groups: 1) Brain-injured followed by 10-min. of hypotension; 2) 10-min. hypotension only; 3) brain-injured only; and 4) control. We evaluated motor deficits with four tests: performance traversing a flat narrow beam, the number of footfalls on a pegged narrow beam, the number of foot-faults on a grid platform, and a forepaw preference test. The results indicate the animals that received brain injury and were made hypotensive performed significantly poorer on the flat beam and the grid than the normotensive brain-injured rats or the rats subject to hypotension. Experimental brain injury followed by hypotension has a demonstrated deleterious effects on motor behavior, beyond that of brain-injury alone.

676.5 PHARMACOLOGICAL ATTENUATION OF PHOTOCHEMICALLI-INDUCED APOPTOSIS IN THE RAT BRAIN. A. Khattar*., D.M. Armstrong and H. Mackey. ASRM, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.

It has recently been demonstrated that apoptotic cell death characterized by intraneuronal DNA fragmentation can occur in a mature brain after ischemic damage (Stroke 1993, 24:2022; J Neurochem. 1993, 61:378; NeuroReport 1994, 5:2661). We have used the technique of intravenous photofocal brain injury, induced by the intravenous injection of the photosensi- tive dye rose bengal and skull irradiation with a 400mW laser, to initiate the apoptotic process in cells in the area surrounding the necrotic core (NeuroReport 1994, 5:2661). Apoptotic cells were identified using the immunocytochemical terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) technique (ApopTag kit, Oncor). In addition, the area of the infarcted tissue was measured in brain slices incubated with 2% triphenyltetrazolium chloride for 15 min at 37°C. Further evidence of programmed cell death was observed using a pharmacological approach and blocking: a) endonucleases by intracerebroventri- cular injection of aurintricarboxylic acid (ATA, 5.10, 20 g/ml); and b) protein synthesis by injecting cycloheximide subcutaneously (2.5 mg/kg). Both treatments significantly reduced the number of apoptotic cells in the area surrounding the necrotic core, suggesting that the apoptotic process involved no protein synthesis. In this study we also blocked gluta- mate receptors by injecting MK-801 intravenously (2.5 mg/kg). MK-801 reduced the size of the phototoxemic lesion and decreased the number of TUNEL-positive cells. Our results are consistent with the hypothesis that glutamate receptor activation (probably caused by the release of glutamate from the lesion core) may trigger delayed apoptotic cell death. Treatments targeted at halting this process might provide neuroprotection in post- stroke or post-traumatic brain pathologies.

676.6 ASTROCYTE INJURY: THE ROLE OF EXTRACELLULAR CALCIUM. B.A. Rizgalińska, S. Liang, and F. E. Ellis*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond VA 23298.

Although considerable evidence supports a role for elevated intracellular calcium ([Ca2+]i) in neuronal damage associated with traumatic injury, mechanisms which mediate traumatic injury in the astrocyte are less clear. Using an in vitro model of cell injury (Ellis et al., FASEB J. 1990, 4:1167), we investigated the role of Ca2+ influx in astrocyte injury. Neonatal rat cortical astrocytes were cultured in silicate-bottom wells. Stretch injury was inflicted by a Cell Impactor (3G, 4 Hz, 0.2 mm) that delivers a regulated pressure pulse of air to the silicate membrane, resulting in membrane deformation and stretch injury. For these experiments, silicate membranes were subjected to a 50 mic pulse, resulting in a 6.5 mm deformation of the silicate membrane (38% stretch). The extent of cell membrane injury was assessed by uptake of the extracellular iodinated probe (PI) and release of lactate dehydrogenase (LDH). The highest levels of PI staining were observed in cells injured in Ca2+-free or low Ca2+ medium (0.1-0.5 mM) increasing concentrations of extracellular calcium (0.1-2 mM) resulted in decreased PI uptake. Release of LDH was also maximal in Ca2+-free medium, and was also decreased by increasing extracellular Ca2+ ([Ca2+]o). Thus, the extent of injury was actually reduced by elevated extracellular Ca2+. Neither Ba2+, Mn2+ or Zn2+ could substitute for extracellular Ca2+, suggesting that the effect of injury is specific for Ca2+. Experiments conducted with activated astrocytes demonstrated that stretch injury induced by influx of extracellular Ca2+. Maximal levels of [Ca2+]o (10 fold that of unjured controls) were attained by 5 minutes post injury and returned to near basal levels by 2-3 hours post injury. Neither influx of [Ca2+]o nor the ability of increased extracellular Ca2+ to protect cells from stretch injury could be inhibited by nimodipine, CNQX, MK801 or SKP9635. Thapsigargin elevates [Ca2+]i by activation of the capacitative calcium entry pathway regardless of Ca2+ occupation. Pretreatment of astrocytes with 1 jmol thapsigargin for 5 minutes prior to injury significantly decreased astrocyte injury. Thus, elevation of [Ca2+]o reduces stretch injury of astrocytes. These results may be critical to evaluation of the role of calcium in mechanical injury to the nervous system. Supported by NIH NS 27214.
676.6


The goal of this project is to assess the feasibility of controlling muscles in the hindlimb of cats by electrically stimulating the spinal cord, ultimately as a method for restoring mobility to paralyzed individuals. Earlier work in our lab has shown that the lumbo-sacral portion of the spinal cord contains regions in which stimulation activates specific muscle groups in the leg in isolation. We refer to these regions as "activation pools." We report here on the activation pools for the quadriceps muscle group.

For the experiments, the lumbo-sacral portion of the spinal cord was exposed. The pia was dissected and the pial tissue was transected and attached to a transducer to measure quadriceps force. Activities in the remaining main muscle groups were monitored using a combination of force transducers and EMG electrodes. Electrical stimulus pulses were generated and delivered to the spinal cord with a constant force of 17 mN was generated by single pulse stimulation. These results indicate that the quadriceps muscle group can be selectively recruited by electrically stimulating the activation pools in the lumbo-sacral portion of the spinal cord, and that good control of force can be achieved. If, as expected on the basis of preliminary work we have completed, these results can be extended to the remaining muscle groups in the leg, it would be feasible to utilize spinal cord stimulation for restoring mobility to paralyzed individuals.

Mental Illness II

677.1


23 male primary alcoholics with secondary depression (PASD) were compared retrospectively with 23 age-matched males with alcoholism alone. Polysonomography (PSG) and Hamilton Rating Scale for Depression (HRSD) scores at admission & discharge were analyzed (mixed design ANOVA). Significant effects were detected by Schuckit's 1/2 classification system & were excluded for alcohol withdrawal, other Axis I diagnoses, recent drug use, recent delirium tremens, seizures, or other medical disorders. All remained medication-free throughout their admission.

HRSD-17 was significantly worse in PASD than in pure alcoholics. HRSD-24, overall depression, motivation, latency, and % sleep efficiency improved by discharge in both groups. REM duration displayed a trend toward interaction, decreasing slightly in the pure alcoholics as compared to little or no increase in PASD. Delta sleep abnormalities were severe in both groups at admission; like some other sleep abnormalities, they persisted despite clinical improvement.

Studies in primary depression suggest that short REM latency and decreased delta sleep may be persistent trait markers. (Supported in part by NIMH grants MH18399 & MH30914 & by VA Research Service.)

677.2


Alterations in the serotonergic system have been implicated in suicidal behavior and in chronic alcohol consumption. We sought to determine whether binding to 5-HT2a, a presynaptic serotonin receptor, differs between alcoholics who died by suicide (SA, n=12), compared to controls. A total of 22 normal controls (C, n=12) in homogenates from dorsal prefrontal cortex. Each SA and CA were carefully matched based on age, sex, race, and postmortem delay (PMD) and examined together as a triad. Demographic variables did not differ between groups, including age (SA=37.5±4.9; CA=36.6±4.5; p=0.67) and sex (MA=11.3±1.1h; CA=11.2±3.1h; p=0.53). The ratio of men to women was 10:2 in each group. P, pellets were resuspended in 50mM Tris-HCl buffer (pH 7.7) and precipitated (37°C) for 10 min.

The incubation (20 min) of 125I-labelled 5-HT(8.6x10(-10)M) with 

both groups did not differ between groups. Neither Bmax nor Kd correlated with age. Bmax correlated positively with PMI when all groups were examined together (r=0.73, p=0.03, n=24). Kd did not correlate with PMI in any group. The levels of 5-HT, 5-HTA and TRP did not differ between groups (p=0.05). Bmax correlated with 5-HIAA only in the control group (r=0.75, p<0.01). Kd did not correlate with 5-HT, 5-HIAA or TRP. The data suggest that alcoholics are associated with damage to serotonin nerve terminals independent of the serotonergic deficit related to suicide (MH16745, 1A09004).

677.4


Sevral putative, antipsychotic compounds were administered to rats per os as a suspension in HPMC. EEG was recorded from screw elec
trodes chronically implanted, bilaterally, in the skull over frontal and parietal cortices. The EEG signal was quantitatively analyzed in the frequency domain by estimating the power spectrum of one minute epochs of EEG before drug administration and at intervals following drug administration. Mobility, a metric related to the theta frequency, or power in the theta band (4.0-8.0 Hz) was analyzed as a percent of their values before drug administration. Neither HPMC nor haloperidol changed theta mobility or power. Chlorpromazine, clozapine, olanzapine, ritanserin, and ZD3638 decreased theta mobility. Clozapine and olanzapine increased theta power. The common factor among the compounds that decreased theta mobility was prediction of the 5-HT(2a) receptor at the administered dose. The similarity of the effect on theta mobility between a selective 5-HT2 antagonist such as ritanserin and a compound with a much broader spectrum of receptor affinities such as clozapine also suggests that the decrease in theta mobility observed in this study is indicative of 5-HT(2a) antagonism. Theta slowing induced by serotonin antagonism has also been observed in the hippocampus (VanDover, et al., 1990). The in
crease in theta power induced by clozapine and olanzapine can be attrib
tuted to the heavy sedation produced by these compounds. Sedation would decrease the overall tone of the serotonergic system (Truax & Jacobs, 1979) and thereby augment the decrease in theta mobility induced by 5-HT antagonism.


BIOCHEMICAL PROFILE OF BEFLOXATONE, A SELECTIVE AND REVERSIBLE MAO-A INHIBITOR. Ch. Curet*, G. Damielou, N. Aubin, C. Sauvage, N. Sonnet, G. Carier, M. Bernardes-Chaix, B. Scatton. Centre National de Recherche et de Coordination des Veilles à l'AVRIL, 91404 Orsay, France. Befloxatone selectively and competitively inhibits MAO-A in different rat and human brain homogenates (brain) with KI values ranging from 1.9 to 3.6 nM for MAO-A and from 270 to 800 nM for MAO-B. Befloxatone does not interact with a large number of receptors, monoamine transporters or other amine oxidases. The inhibition of MAO-A by befloxatone is fully reversible in vitro (inclusion method) and ex vivo after a single oral administration. In the rat brain, befloxatone inhibits MAO-A with an EC50 of 0.108 mg/kg, p.o. The levels of 5-HT and 5-HIAA in hypothalamus and striatum decrease the levels of their respective metabolites. In vivo microdialysis studies show that befloxatone (0.75 mg/kg, ip) increases extracellular levels of NA (cortex), DA (striatum) and serotonin (cortex) after acute administration. In rat brain sections and homogenates, [3H]-befloxatone labels a single saturable population of binding sites with a KD of 1.3 and 1.5 nM, respectively which has the pharmacological properties of MAO-A. In vitro (brain) and ex vivo (dwarf brain), the binding of [3H]-befloxatone is displaced by tyramine. In the rat brain, a striking correlation was observed between in vitro and in vivo labelling of MAO-A by [3H]-befloxatone. This provides evidence that befloxatone is a potent, reversible, competitive, and selective MAO-A inhibitor. Befloxatone is currently undergoing phase II clinical trials.

CLOzapine selectively blocks a subset of GABA receptors. R.P. Squitieri and A. Saederup, Nathan Kline Institute, Orangeburg, NY 10962. Earlier, we reported that clozapine reverses only half of the inhibitory effects of 1.0 μM GABA on 35S-IAEB-binding to rat brain membranes in vitro, and speculated that this might be due to selective blockade of some, but not all, GABA receptor complexes (Neurochem. Res., 15:1099, 1990). To test the possibility we combined clozapine, a 3 and 5-HT1 antagonist, and 44 other partial reversers, and found 13 which produced some additive or partial antagonistic effects. If 0-4664 and tranylcypromine gaba the latent increase in effects (EC50 values > 310 nM and 70 μM; additive reversal < 2% and 26%, respectively). These results suggest that clozapine blocks GABA receptors while 0-4664 and tranylcypromine block GABA at other sites that are not blocked by clozapine. Clozapine produces both GABA-negative (orcinol, paraxonal EEO) and GABA-positive (sedation, amniolytic, anisomycin, retardation of electrical kindling, blockade of metadrol-induced injection in cerebels) cyclic-GMP effects. The GABA-positive effects may be indirect, and due to selective blockade of certain types of GABAergic disinhibition: for example, clozapine may block one type of GABA receptor on an inhibitory GABAergic interneuron (eg A12272), but another type on an excitatory principal neuron (eg G2272, Henske et al. J. Biol. Chem. 268:27101, 1993). Selective blockade of certain GABA Receptor complexes may contribute to clozapine's anti-psychotic effect.

KYNNUREINE PATHWAY METABOLITES IN CEREBROSPINAL FLUID AND PLASMA OF TOURETTE SYNDROME PATIENTS. P. B. Chappell, G. M. Anderson, W. K. Goodman, H. Price, L. M. Hahn, D. J. Cohen and J. F. Lederman*, Yale Univ. Sch. of Medicine, New Haven, CT 06510. The hypothalamic (TRH) metabolites kynericaine (KYR), kyneric acid (KA), and kynurenic acid (KYN) are neuroactive, having actions at the NMDA glutamate receptor. Durson et al. (1994) have recently reported substantial elevations in plasma KYN in medicated Tourette's (TS) patients. A possible role for KYN pathway metabolites in TS also has been suggested by reports of their motor effects in animals, and by reports of reduced plasma TRP levels in TS (Leckman et al., 1984; Comings, 1989) and reduced basal ganglia levels of TRP in TS postmortem brain (Anderson et al., 1992).

In contrast to the report of Durson, plasma KYN was only slightly (6-21%) and not significantly elevated in the TS group (N=23), plasma KYN was highly correlated with plasma ACTH secretion in the TS group, CSF levels of KYN and QA did not differ between the TS and control groups. However, the group mean level of CSF KA was modestly (18%, p<0.02) increased in the TS group (N=30) compared to the normal control group (N=41). The small increase in plasma KYN may be consistent with an apparent increased stress responsivity in some TS subjects (Chappell et al., 1994). The increase in CSF KA is intriguing given the previous suggestion that TRP metabolism and kynurenic functioning may be altered in TS (Anderson et al., 1992).

LITHIUM DELAYS ONSET OF OUABAIN-INDUCED EPILEPTIFORM-LIKE RESPONSES IN HIPPOCAMPAL SLICES. R S, E-Mallakhi, A. Schurr, R. S. Payne, B. Li, and R. S. Levy*. Deps of Psychiatry, Anesthesiology, and Biochemistry, University of Louisville School of Medicine, Louisville, KY 40292. Lithium pretreatment has been shown to protect dogs from digitoxin-induced cardiac arrhythmias, protect differentiated cultured human neuroblastoma cells (9yo) from ouabain-induced toxicity, and prevent ouabain-induced behavioral changes in rats receiving intracerebroventricular ouabain. To further study this phenomenon in living neuronal systems we examined the effect of lithium on ouabain-induced epileptiform-like population responses induced by electrical stimulation. Male Sprague-Dawley rats (225-275g) were treated with intraperitoneal lithium (2.5 mg/kg) for 10-21 days, then used as the source for 400 μm thick hippocampal slices. Extracellular recording of electrically evoked population spikes were obtained from the stratum pyramidale of the CA1 region using micropipettes filled with artifical cerebrospinal fluid. Lithium treatment alone did not alter population spike response. Ouabain (3.3 μM) reliably produced epileptiform-like responses. Lithium pretreatment significantly delayed onset of ouabain-induced epileptiform-like activity by at least 15 min compared to untreated animals (p<0.05). Our data demonstrate that lithium can attenuate ouabain-induced effects in hippocampal slices. This system may prove useful in the study of the mechanism of lithium action.

ERP STUDY ON DISASSOCIATIVE DYSFUNCTIONS. E. Kirito, & F. Pomponio, K. Jone, and R. Jobs*. Department of Psychiatry and Neurology, University of Science & School of Med., Tokyo 113. Event-related potentials (ERPs) during an auditory "odd-ball" paradigm were studied in six patients with dissociative disorders. The patients showed significant reduction in the amplitudes of P300 during dissociative disorders compared with the levels at remission. The latency of P300 did not change significantly during the lucid periods and after dissociative disorders. In two patients who showed a high incidence of asynchronous mismatch negativity (MMN) during and after dissociative disorders, the amplitudes of P300 increased at remission to a greater extent than in the other four patients who showed a low incidence of MMN. Moreover, there was no significant difference in the levels of the right or left hemispheric ERP in the brain CT scans compared with that in the age- and sex-matched control group. The finding of the change in the amplitudes of P300 suggests that the mechanism of dissociation, which has been considered to be a defence mechanism of hysteria, can be psychophysiological evaluated by ERPs. The amplitudes of P300 might be a state-dependent biological marker of dissociative disorders. The low incidence of MMN and atrophy of ST suggest the possibility of trait-dependent cerebral dysfunction or fragility in dissociative disorders.

SYSTEMATIC CHANGES IN CEREBRAL METABOLIC RATE AFTER SUCCESSFUL BEHAVIORAL TREATMENT OF OBSESSIVE-COMPULSIVE DISORDER (OCD). J. M. Schramm*, P. Stoessel, L. B. Baxter, K. Martin, A. M. Phillips. U.C.L.A. School of Medicine, Los Angeles, CA 90024. Eighteen drug-free subjects with OCD were studied with PET scans before and after four weeks of cognitive-behavioral treatment. Twelve patients demonstrated clinically significant improvement during the study period; six did not. Three main findings emerged: (1) responders to treatment showed bilateral decreases in caudate nucleus metabolism, distributed along the basal hemisphere metabolism (Cd/mb), compared to non-responders. This finding was more robust on the right (p<0.003) than the left (p<0.02). (2) Before treatment there were significant (p<0.002) correlations of brain activity with OCD severity and the caudate nucleus, cingulate gyrus, and the thalamus on the right. After effective cognitive-behavioral treatment these correlations decreased significantly. (3) There was a significant positive rank order correlation between percentage change in symptom severity rating score before and after treatment and the percentage change in orbital cortex/hem on the left (p<0.003).

These findings demonstrate systematic changes in brain function in association with successful cognitive-behavioural treatment.

Anterior capsulotomy, a surgical procedure disconnecting fibers connecting prefrontal cortex and thalamus, has been used to treat severe obsessive-compulsive disorder (OCD) when standard clinical interventions have failed. Reports of this procedure typically include extensive pre- and post- surgical evaluations of psychological and neuropsychological functioning. However, to date, no study has investigated changes in physiological reactivity to emotional stimuli which might result from anterior capsulotomy.

We report here on a case of a 33-year-old patient (WW) with a 25-year history of severe OCD which provided refractory to standard treatments, including multiple drug trials, intensive behavior therapy, and ECT. Prior to undergoing anterior capsulotomy via stereotactic radiosurgery techniques, WW participated in several physiological assessment procedures currently in standard use in the Fear and Anxiety Disorders Clinic at UF. Heart rate, skin conductance, corneal EMG, and eyeblink startle responses were collected during an affective imagery procedure and while the patient viewed a series of affective slides. Prior to surgery, WW showed enhanced startle responding, and increased heart rate, skin conductance and corneal EMG as well as eye blinking, when viewing or imaging fear-related, rather than neutral or pleasant stimuli. These data were presented and contrasted with post-surgical data.


The findings that expansion of triplet repeat is responsible for several neuropsychiatric disease has introduced new concepts in human inherited illness. In such diseases including Huntington's disease (HD), spinocerebellar ataxia type 7 (SCA7) and dentatorubral and pallidodegeneration (DRPLA), Machado-Joseph disease (MJD), earlier age at onset, increasing severity of illness are associated with triplet repeat expansion. Marked expansion of triplet repeat may explain the phenomenon of genetic anticipation. Recently, anticipation has been reported in schizophrenia pedigrees suggesting a possible implication of triplet repeat expansion in schizophrenia. To identify novel triplet repeats containing genes associated with neuropsychiatric diseases, PCR was used to isolate cDNA fragments with triplet repeats. Priming with a dinucleotide 10 CAG repeats and sequences in the phase vector results in several clones with CAG repeats. DNA sequencing and computer data base search to GenBank and EMBL demonstrated that these clones are novel molecules. Northern analysis of these genes is in progress.

MENTAL ILLNESS—DEPRESSION

678.1 LATE ONSET DEPRESSION AND MRI DETERMINED BRAIN VOLUMETRIC MEASURES. A. Kumaret, D. Miller, W. Billier, D. Ewbank, D. Walsh, S. Berns* and J. Sulzer*. University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The purpose of our study was to examine the relationship between the age of onset of the first episode of major depression and global and regional normalized volumetric measures in elderly subjects with Major Depressive Disorder (MDD). Our sample comprised of 26 subjects (7 M, 19 W, Mean age ± SD=75.14±6.6) with late onset MDD — the first episode occurring after age 60. All subjects had Hamilton depression scale scores of 2 or 3 at admission and were free of somatic medical disorders with no evidence of clinical dementia or other brain disease. MRI scans were performed on a 1.5 Tesla GE scanner with head coil (TR=3000, TE=38,00 msec). A segmentation algorithm was used to segment brain parenchyma from CSF and whole brain, hemispheres and frontal and temporal lobes were outlined as previously described (Cowell et al. J Neurosci 1994). Absolute volumes of brain and cerebrospinal fluid (CSF) were normalized using intracranial volume to correct for variations in head size. A multiple linear regression was used to examine the relationship between normalized MRI measures and age of onset of illness while adjusting for the subjects current age. Normalized whole brain CSF and total significant volumes showed significant increases with age of onset of illness, r2=0.48 p<0.05 and r2=0.63 p<0.05 respectively, while frontal lobe volumes decreased significantly as age of onset increased (r=-0.19 p<0.05). These data demonstrate the presence of late onset depression, global and focal measures of atrophy increase with age of onset of illness. They also suggest that neuroanatomical contributions to the disorder may increase with a later age of onset of illness.

678.2 INTRACELLULAR CALCIUM SIGNALS IN DEPRESSION MODELLED BY SINGLE-CELL RECORDINGS OF T-LYMPHOCYTES. C. Caccia*, D. Drobes, S. Duff, F. Buscemi and J.B. Aldehan*. Central Institute of Mental Health, Cell Physiology Laboratory, D-75350 OFFENBACH, Germany.

The aim of the present study was to test the hypothesis of an altered Ca2+ homeostasis in psychiatric disorders and more specifically in depression. To this end, T-lymphocytes served as a peripheral model of CNS neurons because of their similarity in receptor types and membrane properties. Thus, this easily accessible cell system provides a means to delineate cellular events along the signal transduction pathway and to detect possible changes during depression as well as during therapy. [Ca2+]i-measures were performed by fluorescence photometry in single T-lymphocytes of 20 patients with major depression (diagnosed according to the DSM III-R criteria; Hamilton rating scale > 18 at first testing, 16) and of 20 healthy controls matched for age and gender. [Ca2+]i of T-cells was also assessed after patients had been treated for 8 weeks (ii) either with Interpersonal Psychotherapy (IPT)(N=13) or else with pharmacotherapy (N=7).

The Ca2+ response of T-lymphocytes to phorbol ester (30 μg/ml PHA) was markedly reduced during depression. Not only was the percentage of PHA-responsive T-cells lower in depressed patients (29% in contrast to 50% in healthy subjects), but also the maximal [Ca2+]i-signals were altered: longer onset latency, slower [Ca2+]i-rise, smaller area under the curve, fewer Ca2+-oscillations. Upon clinical recovery, all depliant Ca2+-parameters were found to normalize independently of the therapeutic approach. Our results indicate that single-cell recordings make it possible to analyze the dynamics of Ca2+-signals and yield information having clinical implications for the monitoring of cellular functions in depressed patients under therapy. (Supported by the German Research Society and by the Volkswagen foundation. We thank J. Sulzer for laboratory work, M. Fritzschke & P. Gabriel for the supervision of patients).


Rats forced to swim after an initial burst of activity become immobile. The onset of immobility is accelerated on subsequent trials. Immobility has been interpreted as a state of lowered reward for despair which is reduced by administration of anxiolytics between trials. Despite false positives behavioural despair has a claim to validity as an animal model of depression. Four experiments were conducted which together challenge this claim. I. Male Wistar Sprague rats (120g) were immersed in 27°C water for 30 min on four occasions. Activity decreased across trials and was correlated (r=0.49) with a graded loss of core temperature. Experiment 2: Ten rats were immersed in 25°C water on trials 1 and 2 (15 min for trial 1; 5 min for trial 2). Temperature was reversed for trial 3 and reversed again for trial 4. Activity varied with water temperature. Reverse from 35°C to 15°C water produced an increase in activity, and was decreased by a reverse from 15°C to 35°C water.

Experiment 3: Forty one rats were immersed twice in 15, 20, 22, 25, 30, 35, 37, or 40°C water (5 or 6 per group). Activity in the second trial was a quadratic function of water temperature. Only 25°C and 35°C water produced significant 20°C reductions in activity between trials. Experiment 4: Typical, atypical, and non-antidepressants were administered (5mg/kg) between trial 1 and 2 and the rats were immersed in either 25°C or 35°C water. There were no differences in activity on trial 2 as a function of water temperature for saline treated rats. Compounds which reduced core temperature (-3°C or more) increased activity in the second trial over the saline control in 25°C water. This was not true at 35°C. It is concluded that activity in the behavioural despair test reflects behaviour thermo-regulation and compounds tested at 25°C, as in the usual procedure, which produce hyperthermia result in increases in activity associated with cooler water.


In the last ten years the desmopressin suppression test (DST) was used as one of the diagnostic methods for detecting several psychiatric diseases, such as depression. Although in these cases high percent of DST positivity was found, only little attention has been paid to the role of stress in DST positivity. In this study 140 healthy young males were investigated in the time of a stressful life event (day of military induction). One mg desmopressin was given p.o. at 22:00 PM and the next day 8:00 AM a blood sample was taken. Cortisol was measured by radioimmunoassay (RIA) method. The cut off point was 5 μg/dl. All subjects underwent a complete physical and psychiatric check up. This test might give an abnormal DST result. One month later only 4% of the subjects showed DST nonsuppression. The results suggest, that a stress situation alone can cause reactively frequent DST nonsuppression in healthy, young men. 

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678.5
SEROTONIN (5HT) 1A AND 5HT2A RECEPTORS IN PREFRONTAL CORTEX (AREA 10) AND HIPPOCAMPUSS OF SUICIDE VICTIMS WITH MAJOR DEPRESSION. C.A. STOCKMEIER*, G.E. DELLEY, L.A. SHAPIRO, J.C. OVERHOLDER, P.A. THOMPSON, AND H.Y. MELTZER. Departments of Psychiatry and Psychology, Case Western Reserve University, Cleveland, OH 44106.

Several studies in the past decade have yielded conflicting results on altered numbers of 5HT receptors in prefrontal cortex and hippocampus in depression and suicide. In the present study, we examined the hypothesis that 5HT-1A and 5HT-2A receptors were increased in suicide victims with major depression. Samples of right prefrontal cortex (area 10) and hippocampus were collected at autopsy from 20 suicide victims and 19 control subjects. Retrospective psychiatric assessments were conducted for all suicide victims and most of the age-matched control subjects. (7-[3H]-GF-DPAT and [3H]ketanserin binding to 5HT-1A and 5HT-2A receptors, respectively, was measured using quantitative receptor autoradiography. Neither age nor postmortem interval significantly affected these receptor measures. In either brain region, there were no significant differences in radioligand binding between all suicide victims and control subjects, or between a subset of suicide victims (N=11) with major depression (and no psychoactive substance use disorder) and control subjects. In the latter group of suicide victims, no antidepressant or antipsychotic drugs were detected postmortem, and only 2 had recent prescriptions for an antidepressant drug. In conclusion, neither 5HT-1A nor 5HT-2A receptors were altered in prefrontal cortex (area 10) or hippocampus of suicide victims with a history of major depression. Supported by PHS Grants MH45488 and MH41684.

678.6
ANTIDEPRESSANT/ANTIPANIC DRUGS MODULATE GABA A RECEPTOR GENE EXPRESSION. A.N. Bateson1,2, A. B. Greenough1,2 and R.B. Baker3. Departments of Pharmacology and Psychiatry, and Division of Neuroscience, Faculty of Medicine, University of Alberta, Edmonton, Canada T6G 2H7.

Pharmacotherapy of panic disorder can be achieved with a number of classes of drugs that apparently have differing primary sites of action. These include monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and benzodiazepines (BZs) that potentiate GABA A receptor function. Certain MAOIs and TCAs have been shown to have secondary sites of action that result in increased brain GABA levels. It is possible, therefore, that GABAergic transmission underlies the etiology and/or pharmacotherapy of panic disorder. Previous studies have indicated that long-term activation of GABA A receptors results in specific changes in GABA A receptor gene expression. We have chronically exposed rats to representative drugs from each of the therapeutic classes that are used in the treatment of panic disorder to determine whether they produce similar changes in GABA A receptor gene expression. Drugs were delivered via subcutaneously-implanted osmotic minipumps and specific GABA A receptor subtype mRNA levels determined using a multiprobe solution hybridization assay. We have found that BZs and TCAs alter specific GABA A receptor mRNA levels suggesting a novel action of these drugs that may play a role in their therapeutic profile of panic disorder.

Funded by the Alberta Mental Health Research Fund and the Canadian Psychiatric Research Foundation.

678.7
LIMBIC TRH IS DIFFERENTLY AFFECTED BY ANTIDEPRESSANTS (AD) vs SEIZURES. E.L. Lloyd*, A.E. Pekary, M. Chillingar, A. Sattin. Psychiatry, Medicine, Endocrinology & Research Services, W. Los Angeles VA Medical Center & UCLA, Los Angeles, CA 90073.

Electroconvulsive seizures (ECS) are known to trigger bursts of synthesis of pro-opiomelanocortin (POMC) in neural nuclei of several limbic regions, resulting in long-lasting increases of TRH (pGlu-His-Pro-NH2) and its immediate precursors including TRH-Gly. In the seizure model, the magnitude of the TRH-Gly increase is significantly related to the reduced immobility (AD effect) in the forced-swim test (FST) (Ann NY Acad Sci, 739:135, 1994). Bupropion is an effective AD drug and Bupropion (Pro-Leu-Gly-NH2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported (J AffectDisord, 31:227, 1994). We tested both agents in the conventional Forskolin MIF-1 (Pro-Leu-Gly-NH2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported (J AffectDisord, 31:227, 1994). We tested both agents in the conventional Forskolin MIF-1 (Pro-Leu-Gly-NH2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported (J AffectDisord, 31:227, 1994). We tested both agents in the conventional Forskolin MIF-1 (Pro-Leu-Gly-NH2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported (J AffectDisord, 31:227, 1994). We tested both agents in the conventional Forskolin MIF-1 (Pro-Leu-Gly-NH2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported.

678.8

Last year we presented the behavioral data from 4 adolescent female Vervets all of which were cinematographed and bilaterally-destroyed TRH-Gene-Targeted (OX) or control handling (2). Depressive behaviors in the OXs were shown by reduced active grooming and social approach, and increased social withdrawal. Most of these defects were reversed following electroconvulsive therapy, but during subsequent treatment the OXs are lethargic and immobile until they have relapsed since one delivered, then neglected its infant. 11-12 mb post-surgical, the 3 survivors (1 OX died) were sacrificed and 13 anterior limbic, cortical and caudate regions were dissected for extraction and analysis of TRH. Compared with the 2 controls which were similar, TRH in the OBX was moderately or severely reduced in 11 regions. For selected regions, co-plotting these values with OBX-affected behavior scores gave predictable linear plots implicating TRH deficiency in depressive behaviors. Amygdalar TRH was not related to aggression. Support: VA Research Service.

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*Hachio Chemical Co. Ltd., Japan, †Hachio Chemical Research Center, USA.

Increasing evidence implicates abnormalities in the signal-transducing G protein in the pathophysiology of affective disorder. We designed an easy measuring system of human lymphocyte G protein mRNA in order to study G protein-coupled second messenger systems in depressed patients. After synthesizing cDNA library from lymphocyte mRNA, the G protein cDNA was amplified using polymerase chain reaction (PCR) with bioin-labeled specific primers to all subtypes of G protein. PCR product was hybridized to each G protein subtype (G, G, G, G, G, Gb and Gc) specific probe which was immobilized onto microplate. The bioin-labeled G protein probe was washed with alkaline phosphatase-substrate solution was added and color development was monitored by microplate reader. Using this method, we measured each subtype of G protein mRNA in lymphocyte from 15 drug-free depressed patients (severe major depression with melancholia, 4 major depression without melancholia) and 15 normal controls. There was no significant difference of any subtypes of G protein mRNA levels between the patient group and controls.

678.10

The effect of prolonged administration of imipramine (IM), citiplapram (CIT) and (+)-lozapine (OXA) on the level of mRNA coding for dopaminergic (DA) receptors D-1 and D-2 in the rat brain was studied. Drugs (10 mg/kg) were administered twice daily, p.o., for 14 days. Animals were decapitated 2 or 72h after the last dose of the drug. The level of mRNA coding for D-1 and D-2 DA receptors was measured in coronal sections of the rat brain through the nucleus caudatus (NC) and nucleus accumbens septi (NAS) in situ hybridization, using commercially available probes (DuPontNEN), labelled with [35S]DATP. Prolonged treatment with IM (but not CIT or OXA) decreased the level of mRNA coding for D-1 DA receptor, to the same extent in the NC and in the NAS, both shell and core. This effect corresponds well with the decrease of the density of receptors labelled with [3H]-SCH23390. On the other hand, the level of mRNA coding for D-2 DA receptors was significantly up-regulated following chronic administration of IM and CIT (but not OXA) and the effect was more pronounced in the NAS than in the AS-core and AS-shell. Since the AS-core and AS-shell are the binding parameters of H-epinephrine after prolonged administration of antidepressant drugs (ADs) and behavioral studies undoubtedly indicated the potentiation of DAergic transmission by ADs, the present results might indicate the different level of regulation of the mRNA coding for DA receptors. (Supp. by KBN grant #6P20708206, Poland).
PLA, ACTIVITY IS INCREASED IN BLOOD OF PATIENTS WITH SCHIZOPHRENIA AND BIPOLAR AFFECTIVE DISORDER. B.M. Rums, C. H. Hudson, J.C. Erlich, J. Warm and S.J. Kish, Clarke Institute of Psychiatry, University of Ottawa, Canada.

Magnetic resonance spectroscopy studies have suggested above normal turnover of membrane phospholipids in brain of patients with schizophrenia. One possible explanation for these findings is an increase in the activity of the phospholipid catalyzing enzyme phospholipase A$_2$ (PLA$_2$). As a test of this hypothesis we compared the activity of PLA$_2$ in serum obtained from 26 individuals with schizophrenia and 22 individuals having bipolar affective disorder, with a group of volunteers whose neurologically normal controls. PLA$_2$ activity was measured either fluorometrically (Gattaz et al., Biol. Psychiat. 28, 495), or radioactively using whole bacterial membranes as substrate (Albers et al., Pharmacopchir 26, 94). When assayed fluorometrically, serum PLA$_2$ activity in individuals with schizophrenia was significantly increased by 47% compared with control activity (P<0.0001). In addition, a smaller 26% increase was observed in subjects with bipolar affective disorder (P<0.01). However, when the same serum samples were assayed for PLA$_2$ activity using the radioactivity procedure there was no significant difference between the three groups of subjects (one-way ANOVA, P>0.95). Furthermore, there was no correlation between the activity as measured by each assay (r=0.073). Thus, our data support the hypothesis that increased phospholipid turnover in schizophrenia could be explained by accelerated phospholipid breakdown mediated by PLA$_2$.

However, the different results obtained using the two different PLA$_2$ assays, indicate that PLA$_2$ alterations in schizophrenia are probably limited to specific PLA$_2$ subtypes. Supported by the Ontario Mental Health Foundation.


ECS is an effective antidepressant (AD) treatment for severely depressed patients and is often used when AD drug treatments have failed. The ECS-evoked changes in the brain are instrumental in the AD effect of this treatment that have not yet been uncovered. Serotonergic and cholinergic neurotransmission are thought to be involved in the pathophysiology of depression and their modulation may be the basis of AD actions of ECS. Repeated ECS was demonstrated to increase in vivo pontotympanic 5HT$_4$ mediated responses in rats (De Montigny, 1984). We examined ex vivo the effects of ECS on pontotympanic 5HT$_4$ and ACs receptor-mediated responses using single electrode current- and voltage-clamp techniques (M and 5-HT$_4$) responses) in hippocampal slices. Intracellular recordings were performed to measure the ACs-induced M$_4$-mediated reduction of the RFS. Rats received 7 cumulative (20V) or 48 hours resulting in a total treatment time of 2 weeks. Control groups were given either 7 sham convulsive shocks (20V) or only one convulsive shock (80V). All shocks were applied transcerebrally in high intensity currents through subcutaneous electrodes placed at the temples. In the intracellular experiments no clear treatment effects were observed on resting membrane potential, cell input resistance and IC-time. 5HT$_4$-induced outward currents and the concomitant decrease in cell input resistance were similar for all treatment groups. The ACs-induced M$_4$-mediated depolarization and the subsequent desensitization of this response were also not affected. Extracellular experiments revealed no changes in stimulation currents needed to elicit EPSPs with a 2 mV amplitude nor changes in the potency and/or efficacy of ACs to reduce this signal. From these results we conclude that there is no in vivo (or ex vivo) correlate of the ECS-induced increase in the vivo pontotympanic response to 5HT$_4$. Moreover, ECS does not alter M$_4$ and M$_4$ mediated cholinergic neurotransmission in the hippocampus of the rat.
678.17 A RAT MODEL FOR MANIA: LITHIUM PREVENTS OUABAIN-INDUCED PERSISTENT HYPERACTIVITY. R.L. G. G. S. Department of Psychiatry and Behavioral Sciences, Department of Medicine, University of Louisville School of Medicine, Louisville, KY 40292.

We have previously shown that single doses of intravenously (IVC) ouabain alters rat locomotor activity (dose-related increase or decrease). In our ongoing efforts to properly model mania, we have examined several parameters to assess the effect of chronic IVC administration of ouabain on animal locomotor activity and the dose of lithium on these ouabain-induced behavioral changes. Rats were implanted with the ALZET osmotic mini pump filled with ouabain (10^-4 M) or artificial cerebral fluid (acSF) at day 0. Pump continuously delivered ouabain or acSF into left cerebral ventricle for 2-3 weeks. Ouabain (2.5 mg/kg/day) or vehicle injections were initiated either one week before or one week after ouabain pump implantation and continued for 4 or 3 weeks, respectively. Locomotor activity of animals were tested by an activity monitor at days 0, 7, 14, 21, 28 and 35. Our results indicate that chronic IVC ouabain infusion induced a significant increase in horizontal activity, total distance, and number of movements (P<0.05). This hyperactivity persisted for the entire period of ouabain administration. Lithium pre-treatment prevented ouabain-induced hyperactivity, and lithium post-treatment (one week after ouabain administration) eliminated the hyperactivity but not significantly different from control animals. Our study suggests that a dysfunction of sodium, potassium ATPase may be an important mechanism for mania disorder.

678.18 EFFECTS OF OUABAIN ON TRANSMEMBRANE POTENTIAL OF LYMPHOBLASTS FROM MANIC-DEPRESSIVE AND NORMAL INDIVIDUALS. Lisa Fox, R. S. E. M. A. L. Christopher A. Worth, Rena L. Stephen C. Paljärvi, Deptts. of Psychiatry and Pathology, Univ. of Louisville School of Medicine, Louisville, KY 40292.

We have previously noted that lymphocyte transmembrane potential (TMP) is hyperpolarized during mania compared with both euthymic (normal state) biolars, and normal controls. Since the sodium pump, which is important in determining TMP, has been reported to have decreased activity in manic bipolar subjects, we investigated the effect of acute (3 hr) and chronic (4d) ouabain treatment on TMP of lymphoblasts derived from bipolar individuals (n=5) and normal controls (n=5). Relative TMP was quantified utilizing a flow cytometer and argon laser excitation of the cationic dye DIOH, before and after depolarization with gramicidin. Ouabain concentrations of greater than 10^-7 M caused severe cellular disruption in all cell lines. In patient and normal cell lines, respectively, TMP was reduced by 8.7% and 6.1% after 3 hr at 10^-7 M ouabain, (n=5) and by 14.85% and 14.69% after 4 days (n=5). The data suggest cell lines from bipolar patients do not differ from normals in their response to ouabain, and sodium pump inhibition is not likely to be involved in producing the lymphocyte hyperpolarization seen in manic bipolar patients.


YM-35992 (S)-2-[[7-(fluoro-4-indanyl)oxy]methyl]mopholine mono-hydrochloride, has dual mode of action, selective 5-HT re-uptake inhibition (SSRI) with 5-HT antagonist activity in vitro and in vivo. Recent microdialysis studies in uninephrectomized animal with chronic administration with SSRI increases extracellular 5-HT concentration in the rat frontal cortex but not single administration of these. In the present study, we examined the effect of YM-35992 on 5-HT release in the rat frontal cortex using microdialysis technique with representative SSRI, citalopram. Single treatment with YM-35992 markedly increased the extracellular 5-HT concentration in the rat frontal cortex. YM-35992 at a dose of 30 mg/kg(i.p.) gave more than 6-fold increase in the 5-HT level, while the increase induced by 10 mg/kg (i.p.) of citalopram which corresponds to 30 mg/kg of YM-35992 in terms of in vitro-irhibiting activity was less than 3-fold. This result suggests the early onset of action of YM-35992 clinically. We also examined the changes in 5-HT1A receptors in the hippocampus after long-term treatment with YM-35992 and amitriptyline, a representative tricyclic antidepressant(TCA). Repeated treatment with YM-35992 for 14 days produced significant increase in [3H]OH-DPAT binding in the hippocampus. This result indicates that YM-35992, similar to TCAs, could produce 5-HT1A supersensitivity in the rat hippocampus. Although further investigations should be needed, these effects of YM-35992 suggests its potent clinical efficacy.


Previous studies indicate relatively high prevalences of rates of thyroid antibodies in depressed patients compared to normal controls. These studies measured titer of these antibodies using generally outdated, indirect assays, e.g., red blood cell hemagglutination assays. The present study used sensitive methods to quantify the concentrations of thyroid-binding (anti-TG) and antithyroid peroxidase (anti-TPO) microsomal thyroid antibodies in patients with anxiety, depressive, mixed anxiety/depression, post-partum mood disorders, and normal controls. Medically healthy individuals (n=178), without any known history of thyroid disease were studied. Patients with anxiety disorders (n=293), depressive disorder (n=43), comorbid depressive and anxiety disorders (n=22), post-partum depression (n=36), and post-partum women without a psychiatric diagnosis (n=14) were compared to a normal population (n=36) who had no past or present psychiatric disorders. Serum concentrations of T4, T3, and TSH were measured by standard techniques; anti-TG and anti-TPO antibodies were determined using the Nichols Antistadins Chemiluminescence assay. The prevalence of anti-TPO antibodies was greater in women with major depression, anxiety disorder, or comorbid depression and anxiety compared to a normal control population; this finding was particularly striking in women under 40 with comorbid depressive and anxiety disorders. No differences between depressed and non-depressed post-partum patients were detected. Serum TSH concentrations in patients with mood and anxiety disorders were significantly correlated with serum concentrations of anti-TG and anti-TPO. Further studies of HPT axis function in psychiatric patients are warranted. (Supported by NIMH MH-51761.)

NEUROTOXINS III


Pentosan polysulfate (PPS), a polysaccharide mucopoly saccharide, has been shown to exert inhibitory effects on HIV-1 replication and inhibit parasite acute growth factors secreted by a variety of malignant cell lines. Sarumin, another polysulfated chemo therapeutic agent has been extensively studied in our lab as a neurotoxic agent. In this study, PPS (100 µg/ml) was found to induce significant cell death in PC12 cells by 48 hours (84% cell viability), but not to the extent that sarumin (600 µM) caused cell death (9% cell viability). On the other hand, PC12 cells exposed to PPS observed limited neurite outgrowth as compared to cells exposed to NGF (10 ng/ml) or sarumin (300 µM). Western blot analysis revealed tyrosine phosphorylation of the TAK1 receptor (S200) in cells exposed to PPS for 10 minutes and 1 hour. The level of phosphorylation was less than that induced by NGF or sarumin. In DRG explants, PPS was also found to cause an inhibition of neurite outgrowth in a dose-dependent manner. The level of inhibition was similar to that in sarumin treated DRG. Ultrastructural studies of stained sections were performed on PPS exposed to sarumin or PPS for 2, 4, and 6 days. Lamellar inclusion bodies (IB) were consistently observed in sarumin treated, but not PPS treated, DRG. Conclusion. PPS mimics the effect of sarumin at the NGF receptor; at low concentrations, it is an agonist and, at higher concentrations, a competitive and non-competitive inhibitor of IB which is, therefore, independent of the receptor effects. PPS will be useful in dissecting out sarumin's chemotherapeutic activity from its neurotoxic action on DRG (supported by NS 27969).


Young animals exhibit an increased susceptibility to the lethal effects of a neurotoxic insecticide (CNE)-inhibiting insects including chlorpyroxide (CPF), although the mechanism(s) for such sensitivity is unknown. Since previous studies in our laboratory indicate that young rat brain acetylcholinesterase (AChE) is more sensitive to CPF on active metabolite, we decided to characterize the blood ChEs of young (PND 4 to 23) and adult (90 day, n=5) male Long Evans rats. Total plasma ChE activities for the PND 4 and adult rats was 60% AChE/40% BChE in PND 4 plasma and 50% AChE/50% BChE for adult plasma. KC50 (50 min at 26°C) were defined concomitantly for PND 4 and adult plasma AChE from 40% to 50% inhibition, respectively; for CPF Plasma KC50 showed age-related differences: PND 4 = 5.5 mM and adult > 44 mM. (The brain KC50 were virtually identical [4.9 mM at both ages].) KC50 values may be underestimated with adult values are not taken into consideration. Therefore, KC50 were also determined in the presence of 1 mM EGTA showing a reduced, but still significant, age-related sensitivity to CPFoxon: PND 4 = 4.4 mM and adult = 12.7 mM. In summary, the AChE of young and adult brain are similar regarding their in vitro sensitivity to CPFoxon. There is, however, a significant age-related sensitivity to CPFoxon (with or without EGTA) in the sensitivity of plasma AChE to CPFoxon, which partially explains the increased sensitivity of young animals to the anticholinesterase insecticide chlorpyroxide.
679.4 EFFECTS OF FUMONISIN B1 ON BLOOD-BRAIN SPHINGANINE TURNOVER AND FB1 TOXICOKINETICS IN THE DEVELOPING RAT. D. S. Moon and W. Sikker, Jr. Dept. of Pharm. Toxicol., Univ. of Michigan, Ann Arbor, MI 48109.

Fumonisins are toxic mycotoxins that are produced by Fusarium moniliforme in corn. Fumonisin B1 (FB1) has been reported to increase sphinganine (Sa) in kidney, liver and serum from rodents and other mammals. The objective of the present experiment was to elucidate whether blood Sa levels has effects on the brain Sa turnover. FB1 and Sa levels both in brain and plasma were measured by HPLC from postnatal day (PND) 12 rats treated with a single dose of FB1 (0.8 mg/kg or 8 mg/kg). Area under the curve (AUC, 0 to 24 hr) of Sa levels was remarkably higher in the forebrain (124.1 nmol/ml/hr) than in plasma (3.1 nmol/ml/hr) after 8 mg/kg FB1. AUC ratios of brain to plasma Sa concentration curves following acute administration were 37.2 at 0.8 mg/kg FB1 and 39.7 at 8 mg/kg FB1. The AUC ratio of brain to plasma FB1 was 0.03. Observations from plasma FB1 disappearance curves over a 12 hr period indicated that the plasma half life of FB1 in PND 12 rats is 1.2 to 1.7 hr, and the volume of distribution is about 0.2 l/kg. These data indicate that even low bioavailability of FB1 in the brain is sufficient to produce the striking increase of Sa levels in the brain. These data also suggest that elevations of brain Sa levels are related to direct action of FB1 on the brain rather than the transfer of blood Sa into the brain.

679.5 INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR-α SYNERGISTICALLY MEDIATE NEUROTOXICITY VIA NO AND NMDA RECEPTORS. S. Ha* and L. Eilich, P. K. Petersen & C. C. Chen. Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN 55404.

The pro-inflammatory cytokines interleukin (IL-1) and tumor necrosis factor (TNF-α), produced by cells within the brain in a pathogenic role in some neurodegenerative diseases, however, little is known about the mechanisms underlying cytokine-mediated neurotoxicity. Using human fetal neuronal cell cultures, we investigated the effect of these cytokines. Although neither cytokine alone was toxic, IL-1 (20 ng/ml) and TNF-α (20 ng/ml) in combination for 7 days caused marked (P <0.01) neuronal injury, as assessed by increased release (>80% over control) of lactate dehydrogenase (LDH), a marker of neuronal loss. In the presence of TNF-α (20 ng/ml), 1 mg/ml of IL-1β caused maximal neuronal death. The E(LD50) of IL-1β was approximately 300 pg/ml in TNF-α-treated neuronal cell cultures. The neurotoxic effect of IL-1β on TNF-α neurons was synergistic (10 μg/ml) of IL-1β, TNF-α + IL-1α blocked 94%, 97% and 9% of DLDH release, respectively. Blockade of nitric oxide (NO) production with the NO synthase inhibitor was accompanied by marked (P <0.01) reduction (about 45%) of cytokine-mediated neuronal injury, suggesting that NO is partly involved in mediating cytokine-mediated neurotoxicity. Addition of the N-acetyl-D-aspartate (NAD) receptor antagonist to neuronal cells cultured blocked (P <0.01) cytokine-mediated neuronal injury by 50%, suggesting the involvement of NMDA receptors. Thus, treatment of neuronal cell cultures with IL-1β plus TNF-α inhibited (P <0.01) the high-affinity 'IL-glutamate uptake and astrocyte glutamate synthase activity, two major pathways involved in protection against NMDA receptor-mediated neurotoxicity. These in vitro findings may lead to development of new therapeutic strategies for neuronal damage in cytokine-mediated neurodegenerative diseases.


The effects of several metals on the serotonin receptor-channel complex were studied using mouse neuroblastoma (NB1-9) cell lines. The cell lines were maintained in a 1% fetal bovine serum-containing medium. The NB1-9 cells were grown in a 1% fetal bovine serum-containing medium.


Bilirubin, a degradation product of protoporphyrins derived from heme, is a well-known neurotoxin. However, neither the cellular nor molecular mechanisms underlying bilirubin-induced neurotoxicity are known. We now report that low concentrations of bilirubin are toxic to cultured rat cerebral neurons. By contrast, cultured fetal rat cortical or hippocampal neurons are relatively insensitive to bilirubin toxicity. Moreover, bilirubin-induced toxicity of cerebral neurons is associated with the biochemical and morphological features of apoptosis. Bilirubin-induced apoptosis is blocked by RNA and protein synthesis inhibitors, suggesting a gene-mediated mechanism. Gel shift (DNA nuclease) analysis we found that bilirubin activates the transcription factor NF-κB in a concentration-dependent manner, and also in both control and Fas-stimulated cerebral granule neurons and cell-free extracts. Moreover, TPK and TCF, specific inhibitors of NF-κB, block both bilirubin-induced apoptosis of cerebral granule neurons and bilirubin-induced NF-κB activation. We suggest that specific activation of NF-κB is the initial intracellular signal mediating bilirubin-induced apoptosis of cerebral neurons. NF-κB may play a more general role in neuronal apoptosis as well.


The anti-HIV therapeutic didanosine (dDI) has been reported to produce a painful, dose-limiting peripheral neuropathy in HIV-infected patients after chronic dosing. This toxicity is dose-limiting, as the disease itself is complicated. Therefore, the nature of this peripheral neuropathy was examined in a non-HIV infected animal model. Rats (N = 9/group) were gavaged with vehicle (0.0 M), 41.5 or 415 mg/kg dDI, or 41.5 mg/kg indomethacin (INH; as a positive control) twice daily for 20 weeks. Plasma dDI levels peaked at 30 min and were constant over the first 1.5 hr after administration, averaging 1.50 ± 0.69 ng/ml at 0.41 and 1.57 ± 0.04 pg/ml at the low and high dose, respectively. Average INH plasma concentrations during the same time period were 18.5 + 7.21 μM. Schedule controlled operant behaviors, motor assessments, and conduction studies and behavioral pathologies were examined. Mortality was dose and drug dependent: high dDI (44%) > INH (33%) > low dDI (0%) = VEH (0%). Ataxia and seizure activity were first noted in the INH group and increased in both groups. In addition, in the INH group, myelin splitting, wallerian, and extracellular debris, mast cells, and reduced axonal number were observed in sciatic nerve sections. These findings were consistent with the behavioral, clinical, and morphological features of dDI administration, without any nerve conduction or behavioral alterations, but histological analysis revealed myelin splitting and intramyelin edema. Thus, rats chronically dosed with dDI developed peripheral neuropathy, even in the absence of HIV infection. (Supported by NIEHS IAG #01-10517)
679.9
LACK OF PROTECTIVE EFFECTS OF SIGMA LIKANS ON THE INDUCTION OF HEAT SHOCK PROTEIN HSP-70 IN RAT CEREBROSPINAL FLUID NEURONS BY DIZOCILPINE*.
NCNP, Tokyo 187 and NIMH, NCNP, Chiba 272, Japan.

The non-competitive NMDA receptor antagonists such as MK-801 (dizocilpine) and phencyclidine induce a discrete populations neurons in the posterior cingulate and retrosplenial cortex of rat brain. These drugs produce vacuolization and necrosis in these neurons. Sharp et al. (1992) have reported that these drugs induce production of heat shock protein HSP-70, and that antipsychotic drug haloperidol and sigma agent rimacoza protect the induction of HSP-70 protein by dizocilpine and phencyclidine, suggesting a role of sigma receptors in the expression of HSP-70 protein by NMDA antagonists. We studied the role of sigma receptors on the induction of HSP-70 protein by dizocilpine. Dizocilpine (1 mg/kg, i.p.) was injected into female SD rats. The induction of HSP-70 protein by dizocilpine produced the induction of heat shock protein HSP-70 in the posterior cingulate and retrosplenial cortex of rat brain. However, the pretreatment with 4-
PPBP and NE-100 could not attenuate the induction of HSP-70 protein by dizocilpine. Thus, it is unlikely that sigma receptors may play a role in the induction of heat shock protein HSP-70 by NMDA receptor antagonists.

679.11
DOMOIC ACID: ASTROCYTIC, NEURODEGENERATIVE AND BEHAVIORAL RESPONSES.
NCTR/DFDA, ANI/DFDA, Laurel, MD, MD.

Four groups of 6 adult male rats were treated i.p. with domoic acid (0, 0.22, 0.66, or 1.33 mg/kg). Eight days later, after behavioral testing, rats were perfused. Hippocampal sections were stained for neuron degeneration and for glial fibrillary acidic protein (GFA). Only a subset (2/6) of the high
dose group had extensive damage, mainly in CA1. GFA
astrocytes in CA1 were larger in high dose rats than controls (107 ± 26 vs. 53 ± 7 sq microns, p < 0.05, mean ± SEM of 6 rats). The 2 rats with the most extensive lesions were the most hypophagic after dosing, were poor at passive avoidance testing, and exhibited exaggerated startle response, suggestive of the presence of a behavioral hyperreactivity syndrome. There was a smaller, but still dose-related increase in astrocyte size even at domoic acid levels not causing neurodegeneration, "reactive" gliosis, or behavioral effects. Our data indicate correlations between neurohistological and neurobehavioral biomarkers of domoic acid exposure, and emphasize the potential importance of astrocytic response to low-dose exposure.

679.12
APOTOTIC CELL DEATH INDUCED BY THE NEUROTOXIN ETHYLCHOLINE AZIRIDNIUM (APFA4) IN VITRO AND IN VIVO.
Pharmacology and Institute of Neurology, Univ. Vienna, A-1000 Vienna, Austria.

Increasing evidence indicates the significance of programmed cell death for neurodegenerative diseases. Various neurotoxins induce neuronal cell death by induction of apoptosis, at least in vitro. In this study we focused on the mechanisms of cell death induced by the neurotoxin ethylcholine aziridinum (APFA4) in vitro and in vivo. The in vitro effect was compared with that of the aziridinum derivative of N2-chloroethyl-N,N-diethylammonium (DSP4). For the in vivo study male Sprague Dawley rats (400-500g) received stereotaxical infusion of 1 or 2 smd APFA4 into the left and the corresponding vehicle into the right lateral ventricle. Rats were perfused under deep chloral hydrate anesthesia with 4% paraform-aldehyde 1, 2, 3 and 7 days after APFA4 application. Apoptotic features including nuclear chromatin condensation and DNA fragmentation as revealed by in situ nick translation was observed in various parts of the hippocampus, adjacent parietal cortex and septum, with a maximum after 2 to 3 days. Both neuronal and glial cells were affected. For in vivo evaluation human neuroblastoma cells SK-N-MC and human embryonic kidney cells 293 were used. A dose- and time-dependent increase in the number of apoptotic cells resulted from the exposure to APFA4 (50 and 100nM) or DSP4 (10-100 nM) in the medium. Apoptotic changes started after 8 h of exposure and reached a maximum between 13 and 24 h. In addition to light microscopic observations the characteristic features of apoptotic cell death were identified by electron microscopy. Apoptosis was suppressed by inhibition of endonuclease by Zn (800µM) and by the free radical scavenger Tempol (2mM). The data indicate that apoptosis contributes to the neurodegeneration induced by neuropeptides containing an aziridinum moiety and support their use in models for neurodegenerative diseases.

679.13
THE LOCALIZATION OF 3-NITROPROPIONIC ACID (3-NPA) INDUCED NEURONAL DEGENERATION IN ADULT RATS.
NCTR/DFDA, Jefferson, AR 72079-9502.

The localization of neuronal degeneration following chronic treatment with the mitochondrial energy metabolism inhibitor 3-NPA was determined in adult male Sprague-Dawley rats using a silver degeneration method (Nadler-Evenson) and a novel fluorescent technique. Rats were injected intraperitoneally with escalating doses of 3-NPA (5 mg/kg/day to a maximum 30 mg/kg/day; Monday-Friday). Rats were perfused under deep pentobarbital sodium anesthesia with 4% formaldehyde in phosphate buffer (0.1 M, pH 7.4). Coronal sections were stained using silver impregnation technique specific for degenerating axons, terminals and neurons. Although some variability in the distribution of lesions was observed, areas with a concentrating neuronal of the cingulate and insular cortex, patches of CA1 hippocampal pyramidal neurons, a considerable extent of the axons within the ventral thalamus, a variable portion of the caudate nucleus and neurons of the deep nuclei of the cerebellum. We hypothesize that the pattern of neurodegeneration produced by 3-NPA in the forebrain and the cerebellum relates to a selective sensitivity to energy depletion of these regions.

679.14
COMPARISON OF VESTIBULAR DYSFUNCTION AND PERIPHERAL NEUROPATHY FOLLOWING SUBCHRONIC DRINKING WATER EXPOSURE TO 3,5'-IMINODIPROPIONITRILE (IDPN) IN THE RAT.
Toxicoit, CSIC, 08034 Barcelona, Spain and Neurotoxicology Division, EUSA. Research Triangle Park, NC 27711, USA.

IDPN induces neurofilament (NF)-filled swellings in the proximal region of large caliber axons, and degeneration of vestibular sensory hair cells which causes a syndrome of abnormal behavior. Following acute exposure, vestibular toxicity appears at doses lower than those required to induce overt neuropathic effects. The present study compares vestibular and peripheral nerve effects of IDPN following subchronic exposure. Adult Long-Evans rats were exposed to 0, 0.005, 0.05 0.1 or 0.2% IDPN in the drinking water for 15 weeks. Exposure was discontinued after 7 weeks in a group receiving 0.4 % IDPN due to marked body weight loss. Motor activity was assessed at 0, 1, 3, 6, 9 and 12 weeks of exposure, and vestibular function at 0, 1, 2, 4, 6, 9, and 12 weeks. The vestibular reflex responses in the normal root ganglia (DRG) were assessed for pathological changes at the end of the exposure. Effects on body weight, motor activity, vestibular function and vestibular morphology were observed after 0.2 and 0.4 % IDPN. Axonopathic effects in the DRG were obvious after 0.2 %. Thus, during subchronic exposure to IDPN, proximal axonopathies do not require larger dosages than vestibular toxicity, in contrast to acute exposure.
NEUROTOXINS during fluoxetine remained virus death, firmly demonstrating the neurotoxicity of IDDM sera. In this study, we tested whether NGF might protect NB from the neurotoxic effects of IDDM serum. After plating equally into 35 mm dishes, NB were exposed to 10% of either control subject (n=5) or IDDM patient serum (n=5), and, separately, to the serum plus increasing concentrations of NGF from 100 ng/ml to 2 µg/ml. After 72 h the cultures were lifted from the dishes and viable cell counts by trypan blue exclusion were determined. In the presence of NGF, serum cell numbers increased in a dose-dependent manner, even in the face of the most toxic IDDM serum which killed all cells. On average, the optimal dose was 500 ng/ml. The effects of the IDDM sera were only partially reversed by NGF. In order to test whether serum IDDM causes alterations in trk A gene expression, in situ hybridization was performed on NB using a probe (Genentech, San Francisco) specific to trk A. The results indicated that NB mock a trk A RNA and that the expression of trk A receptor gene is increased 2-3 fold in response to IDDM serum, but not in response to control subject serum. In summary, NGF has a close-dependent, partial protective effect on nerve fibers of IDDM cells treated with neurotoxic IDDM serum. The neurotoxic effect is not due to down-regulation of trk A receptor gene. Further studies are required to determine any role of altered NGF/receptor signaling in the neurotoxicity of IDDM serum.

NEUROVIRULENT SIMIAN IMMUNODEFICIENCY VIRUS STRAIN INDUCES APOPTOSIS IN THE CENTRAL NERVOUS SYSTEM. D.C. Adams*, T.M. Dawson, M.C. Zink, J.E. Clements*, D. Hanigan, & S. Comp. Immunol. Microbial. Infect. Dis. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21202. Studies of human immunodeficiency virus type 1 (HIV-1) infection in the central nervous system (CNS) have shown neuronal loss in discrete areas; however, the presence and mechanism of neuronal death, particularly in the case of HIV-1 associated dementia has remained quite elusive. Programmed cell death or apoptosis, has been clearly demonstrated outside the CNS in HIV-1, but has not been firmly established within the CNS. Using a simian immunodeficiency virus (SIV) animal model for HIV-1 CNS infection, we investigated whether SIV infection of the CNS is spatially associated with apoptosis of neurons and non-neuronal cell types. With the aid of an in situ technique for identifying the 3'-OH ends of newly fragmented DNA characteristic of apoptosis, we demonstrate the presence of apoptotic neurons, astrocytes, endothelial cells, oligodendrocytes and microglia. We also observe the co-localization of apoptotic cells with numerous perivascular inflammatory cell infiltrates and SIV-infected cells. Quantitative analysis reveals significantly more apoptotic CNS cells in neuroinvasive SIV strains as compared to strictly lymphocytic strains and SIV negative controls. Our findings of SIV induced apoptosis in the CNS will hopefully lead to a better understanding of sequelae of HIV-1 CNS infection such as HIV-1 associated dementia.

EVALUATION OF THE EFFECTS OF FLUOXETINE ON MOTOR ACTIVITY IN RATS. Bruce Galvez* and Sharon Hovensky. School of Pharmacy, University of Wyoming, Laramie, WY 82071. Akathisia and other motor dysfunctions are side effects associated with the use of fluoxetine (FL). The mechanisms underlying these motor effects remain poorly understood. Acute administration of FL in rats has been suggested as an animal model of akathisia (Teicher and Walker, Neurosci. Abstr., p. 1668, 1993). Our studies were designed to further characterize motor activity effects of FL in rats injected with FL (30mg/kg s.c) using 2 different methods of activity testing. Measures of photocell counts recorded over 24-hour periods of male or female rats (n=6/group) of Sprague-Dawley rats IP injected with FL had less locomotor activity compared to the activity during the exploratory periods than paired saline-control groups. Decreases in activity were also recorded from FL-treated rats during the rest of the day and nocturnal period. Daily FL treatment significantly decreased activity monitors were used to provide several different measures of motor activity of individual rats. Our results show a 6% decrease in activity over a 24-hour period. Treatment with FL generally showed a decreased total distance traveled consisting of non-stereotyped decreases in horizontal and vertical activities compared to controls, but differences were significant only during the first 2 hrs in the apparatus. However, males treated with FL showed an increase in total distance moved after 3 hrs in the units and the activity consisted of stereotyped horizontal activity. Our findings that FL generally decreases most motor activities of female rats seem to be inconsistent with previous reports of FL-increased locomotor activity and "restlessness", which may involve a complex of findings and motor activity in FL. Additional characterization of motor behavior is needed to evaluate the utility of this model of FL-induced motor dysmotronton.

UPREGULATION OF HIGH AFFINITY NEUROTROPHIN RECEPTOR, TRK B-LIKE PROTEIN ON WESTERN BLOTS OF RAT CORTEX AFTER CHRONIC ETHANOL TREATMENT (CET). B. Black, M. Heaslet and D.W. Walker, Dept. of Neuroscience, College of Medicine, Univ. of Florida, Gainesville, FL 32610. We previously reported that the total neurotrophic activity of hippocampal extracts was significantly (25-50%) reduced after 21-28 wks of CET suggesting that the level of one or more neurotrophic factors may be compromised in CET rats (Wright et al., Brain Res. Neurosci. Lett. 18:279-80). Such compromised level of neurotrophic factor(s) (i.e., IGF-I, IGF-II), changes in receptors for the neurotrophic factors, or combination of both may induce the neuronal degeneration observed in the degeneration-dependent neuronal death after CET. To investigate possible changes in neurotrophin factor-receptors, we examined Western blots of rat cortex after 21 wks of CET. After sonication and ultra-centrifugation, the supernatant of crude lysates of individual animals' cortex was subjected to SDS-PAGE, electrotrode to nitrocellulose membrane, incubation with anti-B and alkaline phosphatase-conjugated protein A, and chemiluminescent substrate reaction. The membranes were then exposed to Kodak XAR film. We observed three bands in control rats (n=6), but 17 bands (n=7) appeared to have significantly higher intensity of trkB-like protein at about 145 kD, which suggests up-regulation of trkB in a certain subset (i.e., RDNF, NT-3, 4, or 5, but not NGF) of neurotrophins in cortex. Supported by NIAAA T32 A1-07564, the Med. Res. Div. of the Dept. of VA, and NIH Grants AA02002 and AA01928.

CYTOKINES AND DELIRIUM: AN EXPLORATORY STUDY. J.R. Mach, D.J. Weckel, N.I. Opal, K.M. Reif and J.A. Mortensen. Geriatric Research Education Clinical Center (GERCC) (116), VA Medical Center, Minneapolis, MN 55417. Delirium occurs in 20-30% of older hospital patients and is associated with a mortality rate nearly twice that of controls. Despite its clinical significance, the pathophysiology of delirium is virtually unknown. Delirium consists of behavioral and cognitive symptoms caused by a variety of conditions that are not primary CNS diseases. Cytokines have been found to mediate many disease-brain interactions, such as eating disorders, stress reactions, cancer, and infections. To date, the role of cytokines in delirium has not been studied. This study examined the relationship between delirium and serum levels of selected "pro-inflammatory" cytokines IL-1α, IL-1β, IL-2, TNF-α, and IFN-γ. Twelve delirious patients were recruited from the inpatient wards at the Minneapolis VA Medical Center. Subjects met diagnostic criteria for delirium using the CAM and were age 51 or older. Patients with pre-existing dementia were excluded. Eight non-delirious inpatient controls, each matched for age and illness severity to a delirious subject, were enrolled for comparison. Serum cytokine assays were performed using ELISA kits from R & D Systems. Wilcoxon matched-pairs signed-ranks test for non-parametric distributions was used to analyze the two sets of serum cytokine data. IL-6 serum levels (pg/ml) declined significantly in the delirious group as clinical symptoms improved (mean ± SE = 40.75 ± 12.24 vs. 20.25 ± 7.99, respectively, p < 0.05). Furthermore, IL-4 was significantly elevated in the delirious group compared to matched controls (49.47 ± 12.36 vs. 16.25 ± 5.16, respectively, p < 0.02). Serum levels of IL-1α, IL-2, IL-1β, and TNF-α were below detection limits in delirious subjects and controls. These results suggest that IL-6 may play a role in delirium pathophysiology. Supported by a grant from the Department of Veteran Affairs.

Although, females appear to be more sensitive to the hepatic consequences of alcoholism, to date, there has been no evidence that females suffer more severe structural CNS sequelae of alcoholism. Several groups have used magnetic resonance imaging (MRI) techniques to compare brain structure between alcoholics and controls. These studies have primarily examined males. We compared cross-sectional area of the corpus callosum in a group of 14, hospitalized, alcoholics (mean age = 38.9±6.7 years) with a group of 9 non-alcoholic women (35.9±4.7 years). The corpus callosum and the inner table of the skull were outlined by hand on a mid-sagittal T1 weighted MRI image. There was no difference in intra-cranial area between the alcoholics and controls; however, corpus callosum area was significantly smaller among the alcoholics (717±78 mm² versus 566±105 mm², p<0.002).

When the corpus callosum was divided into four segments of equal length, the reduction in area did not appear to be localized. Alcoholic males did not differ from control males in corpus callosum or intra-cranial area. These results may indicate an increased sensitivity to alcohol-induced brain damage among females.

METHANOL TOXICITY: SPECIES DIFFERENCES IN RETINAL FORMATE OXIDATION. J. T. Ellis, A. M. Flagg, J. J. Snodgrass, and M. Salamaan. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Methanol is the toxic metabolite responsible for the retinal and optic nerve toxicity produced in methanol poisoning. The primary site of formate oxidation and detoxification to CO₂ is the liver. However, there is little information regarding formate oxidation in the retina, an important target tissue of formate toxicity. The purpose of this study was to compare the capacity of human retinal tissue and retinal tissue from a methanol-resistant species, the rat, to oxidize formate. Formate oxidation was measured in vitro in isolated human and rat retina. The oxidation of 14C-formate to 14CO₂ by human and rat retinal tissue followed pseudo-Michaelis-Menten kinetics. The pseudo-Km did not vary between the two species and averaged 2.3 mM. However, the maximum rate of formate oxidation (pseudo-vmax) was 2-times higher in rat retinal tissue (63 ± 8 n mole CO₂/g/min) than in human (29 ± 3 n moles CO₂/g/min). The apparent in vitro metabolic half-life of formate was calculated to be 31 min in rat retina and 54 min in human retina. Humans and non-human primates are uniquely sensitive to methanol toxicity. These data support the hypothesis that species differences in retinal formate oxidation may play a role in the susceptibility to methanol poisoning. (Supported the American Petroleum Institute and NIH grant ES06648).

Catalase oxidation of ethanol to acetaldehyde in fetal brain may contribute to the toxic effect of maternal alcohol consumption. B. Hamby-Mason1, B. Cobb-Alley2 and M. Henderson1.1 University of Texas Health Science Center. San Antonio, TX 78284 and 2 Armstrong Laboratory. Radiological Frequency Radiation Division, Brooks AFB, TX. 78227.

Previous studies documenting oxidative stress in rat fetuses exposed to ethanol (E) in utero have shown an increase in catalase activity (Cat) in this E-enhanced Cat, combined with elevated Cat in fetal neonatal brain, may increase acetaldehyde (AcHO) formation in excess of that in adult. Both Cat and AcHO production from E were assayed in 10% homogenates of fetal, neonatal and adult brain. Alcohols of the homogenates were incubated (60 min) with 20 mM E. AcHO levels (by HPLC) and Cat were determined. Additions of E to adult brain homogenates increased AcHO by 25% above the control (C) and elevated Cat by 34.1%. Pretreatment with Cat inhibitor 3-amino-1,2,4 azide and 3-amino-1,2,4-triazole significantly decreased production of AcHO by E. Neither 4-methylpyrazole, an alcohol dehydrogenase inhibitor, nor isoniazid, a P450 inhibitor, had an effect on the amount of recovered from brain homogenates. Cat was highest in brains from 17 and 19 day old fetuses (17.0±1.27 and 11.40±2.40 S.E. units) and declined to 8.15±0.42 units for day one neonates and to 3.5±14 units for adults. Neonatal brains more rapidly metabolized E to AcHO (739 vs 251% above C) than adult brain. Exposure to E in utero increased brain Cat in day 17 fetuses at 1 and 3 hrs post E by 55.3% and 92.9% respectively, with a 44% increase in AcHO production at 3 hrs. This indicates that high basal levels of Cat in immature brain, combined with E-enhanced Cat increase production of AcHO at a rate above that in adult brain. Thus, enhanced Cat oxidation of E may an important mechanism underlying the toxic effects of maternal E consumption on the fetal brain.

SYMPOSIUM: DEVELOPMENTAL DETERMINANTS OF RETINAL GANGLION CELLS. L. M. Chalupa, UC Davis (Chairperson); R. O. L. Wong, Washington University; A. E. Hendrickson, University of Washington; R. W. Guilley, Oxford University.

This symposium will deal with diverse mechanisms regulating the formation of some of the key features of retinal ganglion cells and their precise patterns of differentiation. Chalupa will discuss correlated retinal activity patterns demonstrated by multi-electrode arrays and optical recording techniques in relation to spontaneous activity and neurotransmitter responsivity of developing ganglion cells. Hendrickson will present results of light and EM studies, as well as immunocytochemical experiments, directed at assessing the factors responsible for the density shifts that give rise to the primates. She will consider such issues as the suppression of rods and blue cones in the foveal region, and the effects of cell migration on the state of synaptic contacts formed prior to the differentiation of the fovea. Guilley will talk about stages of the guidance mechanisms that lead axons into a crossed or an uncrossed pathway at the optic chiasm, and will relate this to ganglion cell classes identified in cats and ferrets.
684.1 

SUB-THRESHOLD SYNAPTIC ACTIVATION OF Ca\textsuperscript{2+} CHANNELS MIGHT ACCOUNT FOR LOCALIZED CA\textsuperscript{2+} FLUX INTO DENDRITES OF HIPPOCAMPAL PYRAMIDAL NEURONS. E. M. Conlogue and H. Johnston. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Pyramidal neuron dendrites propagate action potentials (APs), yet the site of AP initiation, even for dendritic input, appears to be the soma or axon hillock/initial segment (AHIS) (Stuart & Sakumoto, Nature 367:99, 1994). Recently, experimental evidence has indicated that dendrites can generate APs in the absence of a soma or an initial segment, suggesting that dendrites could, in some cases, act as an alternative site of AP initiation. This study investigated whether dendrites, specifically the distal dendrites, could generate APs in the absence of a soma or an initial segment.

684.2 

MECHANISMS OF ACTION POTENTIAL INITIATION IN SOMA, AXON HILLOCK, AND INITIAL SEGMENT OF PYRAMIDAL NEURONS. E. M. Conlogue and H. Johnston. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Pyramidal neuron dendrites propagate action potentials (APs), yet the site of AP initiation, even for dendritic input, appears to be the soma or axon hillock/initial segment (AHIS) (Stuart & Sakumoto, Nature 367:99, 1994). Recently, experimental evidence has indicated that dendrites can generate APs in the absence of a soma or an initial segment, suggesting that dendrites could, in some cases, act as an alternative site of AP initiation. This study investigated whether dendrites, specifically the distal dendrites, could generate APs in the absence of a soma or an initial segment.

684.3 

A STABLE-STATE, NODIMINE-SENSITIVE CALCIUM CURRENT ACTIVE AT REST IN HIPPOCAMPAL CA1 DENDRITES. D. Johnston*, J. C. Magee, B. R. Christie, and R. B. Avery. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The dendritic calcium current in CA1 pyramidal neurons expresses a number of different types of voltage-gated channels, including Na\textsuperscript{+} and Ca\textsuperscript{2+} channels. These channels are involved in the generation of action potentials and in the regulation of neuronal excitability. The stability of these channels is important for the maintenance of neuronal function. The study investigated the stability of the calcium current in CA1 pyramidal neurons, providing insights into the mechanisms that regulate neuronal excitability.

684.4 


Repetitive firing behavior of phrenic motor neurons (PMNs) in the newborn rat was examined to elucidate the interplay between intrinsic neuronal properties and synaptic inputs. Afterhyperpolarizations (AHPs) were observed in PMNs before and after synaptic stimulation in vivo. The AHPs were attenuated by exogenous stimuli, suggesting a role for extrinsic inputs in the regulation of PMN activity.

684.5 

SPACIAL AND TEMPORAL INTEGRATION OF SYNAPTIC INPUT IN RAT CEREBELLAR PURKINJE CELLS IN VITRO. D. Hoek, B. Antkowiak, and A. Burst. Max-Planck-Institut f"ur biologische Kybernetik, Speyerstr. 10, 70569 Tubingen, Germany.

We investigated spatial and temporal integration of synaptic input in cerebellar Purkinje cells. Acute sagittal slices (100 \mu m thick) were prepared from the cerebellum of 2 week old rats according to standard procedure. Somatic whole cell patch clamp recordings were established under visual control using an upright microscope with Nomarski optics. The pipette containing lucifer yellow to allow visualization of the individual Purkinje cells dendritic trees. Two small diameter bipolar pipettes were used. The electrodes were filled from glass tubing (tip diameter > 20 \mu m) and used to activate presynaptic fibers terminating on the recorded cells' dendrites. With the extension and geometry of the Lucifer-Yellow stained dendrite being well visible the stimulating electrodes were positioned at different remote sites of the dendritic tree such that synaptic input elicited by electrical stimulation would activate synapses on two dendritic branches of the dendrite. Synaptic inputs and potentials elicited by the stimuli were measured using voltage clamp and current clamp conditions, respectively. Stimuli were applied through each of the electrodes separately and in combination. The voltage and current responses were recorded while varying delays between the two stimuli were given in order to investigate temporal summation. Finally both electrodes were activated synchronously.

Under rapidly ramped conditions somatic current evoked synaptic currents summed up linearly. Preliminary results obtained under current clamp conditions suggest that excitatory synaptic potentials elicited at two independent dendritic branches also sum linearly.

A Purkinje cell was anatomically reconstructed and a compartmental model of the cell was implemented on a computer. The experiments were repeated in the simulated cell.
COINCIDENCE DETECTION OF SYNAPTIC INPUTS AND SPIKES BY INDIVIDUAL SPINES REVEALED BY CALCIUM IMAGING WITH TWO-PHOTON MICROSCOPY

R. Titter* and W. Denk, AT&T Bell Laboratories, Murray Hill, NJ 07974.

Most synaptic connections in the central nervous system occur on dendritic spines, whose functional properties are poorly understood because of experimental difficulties resulting from their small sizes (~1 μm). We have taken advantage of the improved tissue penetration provided by twophoton excitation of visible fluorophores with infrared light (Denk et al., Science 248: 73-76, 1990) to image calcium concentration in spines from CA1 pyramidal neurons in slices of rat hippocampus. Cells were filled with the calcium indicators Calcium Green 1 or SN, using whole-cell perfusion. Laser scanning was performed using a modified confocal microscope with a mode locked Ti:Sapphire laser as the source of pump light.

Focal subthreshold synaptic stimulation produced calcium accumulations that were restricted to isolated spines, showed stochastic failures, and were abolished by post synaptic blockers. Similarly, discrete calcium accumulation localized to isolated spines were seen in spontaneous calcium accumulations elicited by high [Ca++] and low (50 μM) ACSF; conditions that enhance spontaneous transmission release. In contrast, single spines induced fast-peaking calcium accumulations in spines throughout the cell. With 2 μsec time resolution, we observed no appreciable delay between spike-induced calcium accumulation in spines heads and nearby dendrites. Finally, the simultaneous pairing of spikes with synaptic stimulation was frequently cooperative resulting in calcium accumulations that were supranormal, i.e. larger than the combined responses to the individual stimuli.

Taken together, our results strongly suggest: (i) the existence of voltage-sensitive calcium channels in the spine heads; (ii) their antidromic activation by the action potential; (iii) that individual spines are calcium compartments and (iv) that they can individually signal the coincidence of the input and output of the neuron, thus serving as basic functional units of synaptic integration.

(Supported by the Office of Naval Research and AT&T Bell Laboratories)

684.9

AT-SOURCE KINETICS OF GABA RECEPTOR MEDIATED IPSCS IN DENTATE GRANULE CELLS. L. Sil полиг*, D. R. Stimers* and M. Dohy†

Dept. of Anatomy and Neurobiology, UC Irvine, CA; "Salk Institute, La Jolla, CA; "Division of Neurology, UCLA, Los Angeles, CA.

Principal cells receive their GABergic inputs from multiple interneuron types which terminate in mutually exclusive domains along their longitudinal axis. In the dentate gyrus both somatic and dendritic layers contain several GABA receptor subunits and an abundant GABAergic innervation. We used whole-cell patch clamp and computational techniques to find out whether there are differences in the properties of the GABergic inputs located on different parts of cells. IPSCs were recorded from adult rat granule cells in the presence of 10 μM CNQX and 40 μM D-AP5, with 130 mM Cl-, 10 mM HEPES, and 2 mM MgCl2-containing electrodes.

Minimal stimulation at various distances from the somatic recording site showed that both proximal and distal inhibitory fibers are capable of releasing GABA, and that the released transmitter activates functional GABA receptors. In addition, these experiments demonstrated that the further the fiber was from the soma the IPSCs were generated, the slower and smaller they became. When spontaneous events were examined in the presence of TTX (minispike or miniPSCs), the 10-90% rise times of the events ranged from 0.1 to 10 ms. How are miniPSCs with such different kinetics generated? Compartmental modeling of a typical granule cell showed that the kinetics of distal miniPSCs at source are sensitive to the kinetics of the somatic miniPSCs. This suggests that activation of somatic and dendritic GABA receptors evokes distinct events at source kinetics. However, when the bulk (≥50%) of the dendritic tree was removed, the distribution of 10-90% rise times still showed an unchanged low (<5%) percentage of events with extremely slow kinetics (3-10 ms), indicating that such slow events can be generated at proximal site.

These findings suggest that in granule cells most distal and proximal synapses contain GABA receptors with similar kinetics, however, they also indicate the possible presence of a small number of synapses with distinct GABA receptor composition and kinetics.

684.11

15R-ACPD INCREASES INTRACELLULAR CALCIUM LEVELS IN RAT DORSOLATERAL SEPTAL NEURONS. J. Zheng and A. Conde. Roche Inst. of Molecular Biology, Nutley, NJ 07110.

15R-ACPD causes membrane depolarization and burst firing in rat DLSN neurons, and results in long lasting enhancement of synaptic transmission (Zheng & Gallagher, Neuron, 9, 163-172, 1992). One possible mode of action is an increase of intracellular calcium levels by releasing calcium from IP3-sensitive stores due to activation of the metabotropic receptor coupled to phospholipase C. In the present study, we measured intracellular calcium levels using fluorescent imaging techniques in a slice preparation. Fura-2 was loaded into rat DLSN neurons through the microelectrode. [Ca++] was measured by 350/365 ratio imaging.

Superfusion of 15R-ACPD depolarized the fura-2 loaded DLSN neurons and triggered burst firing in the soma. During the repolarization phase of the burst firing the dendritic calcium signals returned to baseline level, while the calcium level in the soma was still elevated. [Ca++] changes during ACPD bursts were much larger than those produced by glutamate in normal saline. Since the enhancement of spike-triggered calcium increases by 15R-ACPD is accompanied by a prolonged, depolarizing plateau which is thought to be due to a calcium spike, this action of 15R-ACPD is likely due to an enhancement of calcium influx through voltage-gated calcium channels. However, the [Ca++] change during bursts was reduced by glycine (200 μM) and NMDA (5 μM), suggesting that a large part of calcium increase is due to release from internal stores. Thapsigargin has no consistent effects on basal calcium level. On the other hand, ryanodine (20-100 μM) did not reduce calcium increase elicited by 15R-ACPD-induced bursting. Our data suggested that the rise of [Ca++], associated with ACPD-induced bursting, is due to both calcium influxes from internal stores. (Supported in part by Jeanne E. Kemper Scholarship to F.Z.)

684.10

RECOMBINANT GEPHHRIN FORMS FILAMENTOUS IN VIVO WHICH BIND THE GLYCINE RECEPTOR β SUBUNIT AND GABA receptors β SUBUNIT. J. Kirch*, H. Betz

Dept. of Neurochemistry, Max-Planck-Institute for Brain Research, D-60528 Frankfurt, Germany.

The peripheral membrane protein gephrin was originally identified by co-purification with the inhibitory glycine receptor of rat spinal cord. Expression of this polyepitide in spinal neurons is essential for the formation of post-synaptic glycine receptor clusters. Gephrin is widely expressed throughout the synaptic regions of the central nervous system and co-distribution with glycine- and GABA receptor could be demonstrated. Since both receptor types are perineuronal receptor complexes composed of several subunits we investigated by heterologous expression in combination with electron microscopy and immunocytochemistry which receptor subunits can interact with recombinant gephrin. We found that recombinant gephrin forms filamentous structures (6nm) in HEK 293 cells which bind glycine- and GABA receptor β subunits. Therefore, we conclude that gephrin-mediated anchoring at synaptic sites may depend on the subunit composition of the respective neurotransmitter receptors.

684.12


Brain stem neurons in the nucleus tractus solitarius (NTS) from rats are well suited for patch-clamp analysis of synaptic currents as their passive electrical properties are characterized by a single membrane time constant of 2 ms and an input resistance in the GΩ range. The release of glutamate from receptor mediated synaptic transmission was investigated in paravascular NTS neurons by performing patch clamp recordings in this region. It was found that during the decay phase of the evoked AMPA receptor mediated synaptic responses, we found that fast (t1/2 = 8.5 ms, τm = 0.6 ms) and slow (t1/2 = 0.3 ms, τm = 2 ms) release of glycine was observed in different subregions of the NTS. The slow decay kinetics were also found for spontaneous and miniature EPSCs investigated in 1 μM TTX. The slow decay kinetics did not result from dendritic filtering. Also, it did not reflect the deactivation kinetics of the post-synaptic AMPA receptors, which were also investigated by a 5 Hz application of glutamate to outside - out patches pulled from the same cells. Comparison with fast EPSC decays in other systems showed that the slow component reflects responses from the prolonged presence of glutamate during the synaptic transmission process.
685.1 A NOVEL PROTEIN (HAP-1) ENRICHED IN BRAIN INTERACTS WITH THE HUNTINGTON'S DISEASE PROTEIN X. L. Li,1,2,3* E.H. Li,1 A.H. Sharp,1,2 P. Nueslein1, G. Schilling1, S.H. Snyder1 & C.A. Ross1,2. Laboratory of Molecular Neurobiology & Department of Psychiatry. 2. Department of Neuroscience Johns Hopkins University School of Medicine Baltimore, MD 21205 Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded glutamine repeat in a gene termed IT or Huntington's. HD is characterized by degeneration of the caudate, putamen, and ventrolateral thalamus. Genetic linkagemapping studies indicate that HD is caused by a mutation in the gene that encodes the huntingtin protein. This protein is highly expressed in brain, and recent evidence suggests that it may be involved in the selective brain pathophysiology of HD.

685.3 CELLULAR LOCALISATION OF THE HUNTINGTON'S DISEASE PROTEIN AND DISCRIMINATION OF THE NORMAL AND MUTATED PROTEIN. Patricio Saavedra1,2, Yvon Troppier1,2, Delier Dayes1, Georges Immers1, Isabelle Ane1, Christian Weber1, Xues Ai-Jing1, Raimund G. Burch1 and Jean-Louis Mandel1,2. 1GMC, 67404 Ilkirch, France and 2INSEERM U289, 76501 Paris, France. Huntington's disease (HD) is a dominant neurodegenerative disorder characterized by involuntary movements (chorea), personality changes and dementia. It is caused by an expansion of a polyglutamine coding CAG repeat in a gene of unknown function. The wide expression of the transcript does not correlate with the pattern of neuropathology in HD (neuronal death in the striatum) and the HD gene product (huntingtin), we have developed monoclonal antibodies raised against four regions of the protein. These monoclonals detect specifically huntingtin as a ~350 kDa protein in human and mouse non-neuronal cell lines and in mouse and human non-neuronal cell lines.

685.4 TOWARD AN UNDERSTANDING OF TARDIVE DYSKINESIA: CHRONIC HALOPERIDOL ENHANCES NMDA TOXICITY IN MOUSE STRIATUM. L. Tanaka1 and G. Bonventolin. Research Laboratories of Schering AG, 13245 Berlin and Division of Pediatric Neurology, St. Louis Children's Hospital, St. Louis, MO 63110 Chronic treatment with neuropsychiatric drugs leads to the development of tardive dyskinesias (TD) in a large proportion of patients. The neurotransmitter dopamine receptors explains neither the onset nor the persistence of TD. The nature and the location of the mechanisms in the basal ganglia that are responsible for the development of TD and irreversibility of the symptoms remain unresolved. In patients subjected to chronic treatment with neuropsychiatric drugs who develop TD concentration of glutamate is increased in CSF. Therefore we examined whether acute or chronic systemic treatment with haloperidol (10 mg/kg, i.p. for 21 days) increases levels of glutamate and N-Methyl-D-aspartate (NMDA) in the mouse striatum. Glutamate (1 μM) and NMDA (1 μM) were microinjected into the striatum of NMDA mice, 20-25 g, subjected to acute or chronic treatment with haloperidol, and stereotaxic injections were done at the damage was performed 24 h later. In mice subjected to single systemic administration of haloperidol, neurotoxic-dose of either glutamate or NMDA induced no damage in the striatum, whereas mice subjected to chronic treatment with haloperidol (5 and 10 mg/kg for 21 d) both glutamate and NMDA produced pronounced excitotoxic lesions in the striatum in a dose-dependent manner. These data indicate that long-term treatment with haloperidol might lead to excitotoxic action of glutamate and NMDA. Such observations form the base for the hypothesis that chronic treatment with neuropsychiatric drugs may change the balance between neurotransmitters in the basal ganglia in such a way that even physiological (non-toxic) concentrations of glutamate may become neurotoxic and acting via NMDA receptors lead to neuronal damage in the mouse striatum. The hypothesis of a weak glutamatergic toxicity after termination of neuroleptic drug offers alternative explanation for the evolution and persistence of the symptoms of TD.
685.7 NEUROPROTECTIVE STRATEGIES FOR TREATMENT OF LESIONS PRODUCED BY MITOCHONDRIAL TOXINS: IMPLICATIONS FOR NEURODEGENERATIVE DISEASES. M. T. B. Holt*, M. T. Matthews, D. R. Henshaw and J. P. Schult. Neurochemistry Laboratory, Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Neuronal death in neurodegenerative diseases may involve energy impairment leading to secondary excitotoxicity, and free radical generation. Possible therapeutic treatments of neurodegenerative disorders for diseases therefore include glutamate release blockers, excitatory amino acid receptor antagonists, agents to improve mitochondrial function and free radical scavengers. In the present study we examined whether these strategies either alone or in combination had neuroprotective effects against striatal lesions produced by mitochondrial toxicity. The glutamate release-blockers andrographolide and BW1003C87 significantly attenuated lesions produced by intrastriatal administration of 1-methyl-4-phenylpyridinium (MPP\(^+\)). Laminogignic significantly attenuated lesions produced by systemic administration of 3-nitropropionic acid (3-NP). Memantine, an N-methyl-D-aspartate (NMDA) antagonist, protected against malondialdehyde induced striatal lesions. We previously found that coenzyme Q10 exerted dosedependent neuroprotective effects against malondialdehyde. It is therefore possible that a combination of agents which act at sequential steps in the neuroprotective cascade can produce additive neuroprotective effects.


Following the discovery that patients with familial amyotrophic lateral sclerosis have a gene defect at a locus that encodes superoxide dismutase 1 (SOD1), several groups have developed SOD1 transgenic mice and have observed that these mice at 5 to 8 wks of age display spontaneous onset of paralysis and motor neuron degeneration, which is consistent with the pathology in cell bodies and processes and swelling of the proximal axonal segment. Because other lines of evidence have implicated an excitotoxic mechanism in motor neuron degeneration in ALS, the question arises whether the pathological changes described in the SOD1 mutant mice are consistent with an excitotoxic process. To address this question, we have undertaken studies aimed at quantitatively and accurately describing the degree of excitotoxic degeneration of spinal motor neurons. The excitatory amino acid agonists, DL-homocysteic acid (DL-HCA) or kainic acid (KA), when given systemically to infant rats or when applied directly to the exposed lumbar cord following posterior laminectomy of 21 day old rats, cause excitotoxic degeneration of motor neurons. DL-HCA is a mixed agonist that acts at both NMDA and non-NMDA receptors whereas KA acts only at NMDA receptors. The pathological changes in motor neurons following either DL-HCA or KA treatment were studied by both light and electron microscopy and consisted of intracytoplasmic vacuole formation and dark cell changes in which the entire motor neuron, except the vacuoles, gradually became condensed and shrunken. These changes occurred within the first 2 hrs after exposure to the excitotoxic and were accompanied by changes in the proximal axonal segment consisting of swelling of the proximal myelin sheath with accompanying changes in axoplasmic content. Degenerative changes were not distal parts of the axons accessed over a 12 to 24 hr period. We conclude that on the basis of purely morphological criteria, the spontaneous degenerative process affecting motor neurons of SOD1 transgenic mice is consistent in appearance and time course with degeneration induced by exposure to excitotoxic agonists. Supported by AG 11355 and RSA M138944 (GW).


We have generated several lines of transgenic mice expressing human (Hs) superoxide dismutase I (SOD1) with a mutation (glutamine 37 to arginine - Q37R) that causes familial amyotrophic lateral sclerosis (ALS). These animals develop motor neuron disease; the age of onset, duration, and age at death are strongly dependent upon the dose of the mutant protein. Several investigations have shown that expression of the procoenzyme Bcl-2 can protect neural cells from a variety of insults. To determine whether Bcl-2 is protective against the injury caused by mutant SOD1, transgenic mice expressing the G37R mutant were mated to mice expressing human Bcl-2 driven by the human metallothionin-N (NL-F)-promoter. Heterozygous animals were selected by 5-10 times the level of endogenous mouse Bcl-2 in these mice. In the lines of mice expressing the highest level of mutant SOD1 (G37R)(42), little if any delay in the age of onset of disease was observed in the presence of elevated human Bcl-2. We are presently examining additional parameters, including the duration of the disease and the pathological appearance of affected neurons. Whether Bcl-2 will alter the age of onset or duration of disease in animals with a lower dose of mutant SOD1 is also being examined.

685.11 POTENTIAL INVOLVEMENT OF THE PROTEIN KINASE C PATHWAY IN HIV-1 ASSOCIATED NERVOUS SYSTEM ALTERATIONS. T. Wys-Conn*, E. Madliah, S. R. Troger, H. S. Lee and L. Muck. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, and Dept. of Neurosciences and Pathology, USCD, La Jolla, CA 92039-0634.

HIV-1 associated central nervous system (CNS) disease involves neuronal damage and prominent reactive astrocytosis, the latter being characterized by strong upregulation of the glial fibillary acidic protein (GFAP) in astrocytes. In a previous report, we showed in transgenic (tg) mice expressing the HIV-1 envelope protein gp120 in the CNS (Togasaki et al., Nature 376:188-193). Because alterations of astrocyte functions could contribute to neuronal impairment we compared brains of tg120 and tg210- transduced C6 astrocytoma cells with controls, and found that gp120 induced a prominent elevation of steady state GFAP mRNA levels, due primarily to transcript stabilization. Increased levels of GFAP mRNA were also induced in non-transfected C6 cells by exposure to recombinant gp120. Gp120 effects were significantly decreased by inhibitors of protein kinase C (PKC) but not by inhibitors of protein kinase A (PKA) or calmodulin. gp120 in transduced C6 cells and in the CNS of gp120 transgenic mice. Further, brain tissue from patients with HIV-1 encephalitis and from gp120 transgenic mice showed increased GFAP mRNA levels. Taken together, these results indicate that gp120-induced increases in PKC activity may contribute to the gliosis seen in gp120 transgenic mice as well as in HIV-1 infected mice and raise the question of whether regulation of signal transduction pathways represents a general mechanism of HIV-associated pathogenesis.

685.12 NEURONAL CELL DEATH IN PRION DISEASE. H.A. Kretzschmar*, D. R. Brown, A. Gilmore, J. H. Wiltfang. Institute for Neuropathology der Universit\(\text{"}\)t G\(\text{"}\)ttingen, 37075 G\(\text{"}\)ttingen, Germany.

Neuronal degeneration and cell death are among the most pressing features of prion diseases, the mechanisms of which are onlly poorly understood. Our studies so far have been focused on two questions. First, what mechanisms are involved in neurotivity of the prion protein (PrP\(_{\text{Sc}}\))? Second, what cells is death in prion diseases mediated by mechanisms of apoptosis? Neuronal cell death in prion diseases was studied in three experimental systems.

1. DNA fragmentation and morphology of cell death in mice infected with the scrapie strain 79A.
2. The effect of P1P 106-126, a synthetic peptide corresponding to amino acids 106 to 126 of PrP, on cultures of cortical cells from embryonic day 16 mice. The effect of P1P 106-126 on cultures of cortical cells from embryonic day 16 PrP\(_{\text{Sc}}\) mice (PrP-knock out mice).

Cell death in the 79A scrape strain 79A mice was studied using in situ end labeling (ISEL), and light and electron microscopy. Our results show that apoptosis occurs in an experimental scrapie sample in vivo. In this system apoptosis is observed particularly in the outer nuclear lamina and the gramine cell layer of the cerebellum. In cell culture experiments we showed that P1P 106-126 is toxic to cortical cells from embryonic day 16 mice. P1P 106-126 has no neurotivity effect on cortical cells from this source. This is due to differences between neurons from normal and from knock-out mice observed by us, since using patch-clamp analysis we were unable to confirm differences in the synaptic transmission between knock-out neurons in the cerebellum. In addition, our results show that the neurotivity of P1P 106-126 can be blocked with MK801 and verapamil indicating that it is mediated by an intracellular Ca\(_{\text{2+}}\).
686.1
SENSORY TRANSDUCTION IN THE VOMERINAL ORGAN: IONIC CURRENTS OF DISASSOCIATED MOUSE RECEPTOR NEURONS. L. R. Luman and D. P. Cory*. Howard Hughes Medical Institute and Massachusetts General Hospital, Boston, MA, 02114.

We are investigating the mechanism of sensory transduction in the vomeronasal organ (VNO). The VNO is located at the base of the nasal cavity and contains chemosensitive neurons that are thought to respond to pheromones. While signaling pathways have been elucidated for olfactory transduction, the mechanisms for the VNO are not well characterized. The main goal of our research is to identify how transduction may be different in the two systems. For example, the site of transduction in olfactory neurons - are not present on VNO neurons; VNO neurons instead have "bead-on-wire." We have developed a protocol for preparing dissociated mouse VNO neurons that yields high numbers of relatively intact cells. The diameter of the VNO neuron cell body is 11.2 ± 0.4 μm (n = 12), which is considerably larger than that of olfactory neurons (5-8 μm). A dendritic process is evident that ranges in length from 2 to more than 30 μm and ends in a tuft of microvilli.

As a first step towards understanding transduction, we are using whole cell patch clamp recording to characterize voltage-activated conductions. In experiments with Ca2+ in the pipette, inward Na+ currents were first evident with voltage steps to -50 mV and reached a peak at +10 mV. The currents were half inactivated by a prepulse to -67 ± 3 mV (n = 5), similar to those for Na+ currents, TTX, at a concentration of 300 μM, blocked 54 ± 9 % (n = 3) of the current. We have also recorded K+ and Ca2+ currents and are in the process of characterizing them.

We have begun to investigate whether VNO neurons express cyclic-nucleotide-gated channels. We have found no positive evidence for the presence of cyclic nucleotide gated channels either by excised patch recording or immediate dialysis with cyclic nucleotides and are in the process of confirming these results.

686.3

Immunochemical localization of glucocorticoid (type II) receptor in the mammalian olfactory mucosa was examined by utilization of an affinity-purifi ed antibody raised against a glucocorticoid receptor protein. Immunoreactivity was associated with the saccular cells of Bowman's glands located in the lamina propria.

In addition, the olfactory nerve axons were also immunoreactive. Within the olfactory epithelium, immunoreactivity for the receptor was also observed, which by location corresponds to saccular cells and/or olfactory receptor cells.

There was no immunohistochemical staining when the antibody for the glucocorticoid receptor was omitted from the primary antibody and the antibody of normal serum.


(Supported by Northwestern University Research Fund and NIH CIDA DC00064)

686.5
ACTIONS OF CARBON MONOXIDE AND CYCLIC GMP ON ODOR RECEPTORS OF THE MAMMALIAN OLFACTORY RECEPTOR CELLS. F. Zafar, G. M. Shepherd, and T. Perry-Zufall. Section of Neurobiology, Yale University School of Medicine, New Haven, CT.

There is increasing evidence supporting the function of carbon monoxide (CO) as a diffusible messenger in the vertebrate olfactory system. CO can act as a potent activator of olfactory cyclic nucleotide-gated (CNG) channels by stimulating CAMP formation in the odor receptor neuron (ORN) and this effect provides a possible mechanism for regulating excitable properties of ORNs. (Leinders-Zufall et al, this meeting). Here we describe the interaction of the CO-induced CAMP pathway with the odor-stimulated G-protein coupled CAMP pathway and tested whether this CO/CAMP system can influence the responsiveness of ORNs to odor stimulation.

Odor responses from single单元 ORNs were obtained using the performed patch technique. This permitted recording of both odor-induced membrane currents and membrane potential changes in the input level of the cell that could be related to action potential discharges at the output level of the cell. ORNs that were voltage clamped to their resting potential responded with transient inward currents to short pulses of odor stimuli. In contrast micromolar doses of CO induced long-lasting tonic inward currents. Recovery from this CO effect was on the time scale of minutes. When we measured-transient currents were tested during the long-lasting CO-induced currents, the odor responses were markedly diminished.

This effect could be mimicked by low doses of 8-Br-cGMP. Under current clamp conditions, 8-Br-cGMP produced a membrane depolarization with an inward rectification potential. Micromolar concentrations of CO induced a long-lasting depolarization that modified the spike discharge pattern induced by odor pulses resulting in most cases.

Our results suggest that the presence of CO leads to several discrete changes in the signalling properties of ORNs which, taken together, strongly influence odor responsiveness of the cells. Supported by grants from NIDCD (F.Z.) and from NIDCD and NIMH, NASA and NIDCD (Human Brain Project) (G.M.S.).

686.7
GLUCOCORTICOID (TYPE II) RECEPTORS IN THE MAMMALIAN OLFACTORY MUCCA. RU 28362 BINDING SITES. J. D. Foster, R. M. Sanchez-Pueyo, and D. J. Sjostrand. Dept. of Otolaryngology, Northwestern University, Chicago, IL 60201, Dept. of Anatomy and Cell Biology, Muhay Medical College, Nashville, TN 37208.

The synthetic corticosteroids are the most frequently prescribed medications for olfactory disorders despite the fact that their mechanism of action within the olfactory system is unknown. Recently, a series of studies was initiated to gain insight into the mechanism of corticosteroid action on the olfactory mucosa. In this current study, we have focused on the binding characteristics of the glucocorticoid (type II) receptor.

The synthetic glucocorticoid RU 28362, which has negligible binding to mineralocorticoid (type I) receptors (Philbert et al, Annu. Meet. Endocrine Soc. 546:8-17, 1983, p. 335), was employed to identify high-affinity glucocorticoid (type II) receptors in the olfactory mucosa. By Scatchard plot analysis, the Kd of the [3H]-RU 28362-arylxylophilic receptor complex was 2.2 ± 10^-11 M, while the concentration of binding sites, B_0, was 180 fmol/mg tissue. Time course studies indicated that the binding of [3H]-RU 28362 by the olfactory tissues reached equilibrium within 30 min of incubation at 25°C.

Substantial specific [3H]-RU 28362 binding to the olfactory tissues suggests the presence of glucocorticoid receptors and sites of glucocorticoid action in the olfactory mucosa. (Supported by Northwestern University Research Fund and NIH CIDA DC00064)

686.6

The localization of glycine receptors (GlyRs) and the GlyR-associated protein gephyrin was investigated in the rat olfactory bulb (OB), using immunocytochemistry. The monoclonal antibody (mAb) 6b, which can recognize all of the known GlyR subunits (α1-3, β), produced a diffuse staining in mitral and tufted cells, as well as a punctate staining in the glomeruli. According to previous in vitro and in vivo binding studies (Maldonado et al, EMBO J, 1991), we assume that this staining reflects the presence of the β subunit of the GlyR. Surprisingly, electron microscopy showed that some of the presumed glutamatergic synapses made by the relay neurone terminals onto the dendrites of mitral/tufted cells were immunopositive.

mAβ 2b, which is specific for the strychnine-sensitive α1 subunit, produced no immunoreactivity in the OB, whereas an antibody (P10) recognizing the α1 subunit did not label the α1 subunit. From these data, we conclude that the α1 subunit is not expressed in the OB.

mAβ 7a, which is specific for gephyrin, produced a strong punctate labelling in the external plexiform layer and in the glomeruli. Immunopositive puncta outlined the cell bodies and main dendrites of mitral and tufted cells. Electron microscopy showed that gephyrin is present in the synapses made by local interneurons (periglomerular and granule cells) with the dendrites of mitral/tufted cells. Since many periglomerular and granule cells use GABA as a neurotransmitter, it is likely that in the OB, as in the retina (Sasso-Pugeot et al., J. Comp. Neurol., 1995), gephyrin might be localized to GABAergic synapses (Supported by M.U.R.S.T. 40% and 60%, C.N.R.).
686.7 INTERACTIONS BETWEEN Olfactory Bulb AND olfactory COrTEX IN A NeURAL model OF odor processing. Christiane Linster1, Michael E. Hasselmo2 (1) Dept. Psychol., Harvard Univ., Cambridge, MA 02138 (2) Lab. de Physiologie Neuroérogénétique, CNRS, 69622 Villeurbanne.

Cholinergic mechanisms have been shown to have both excitatory and inhibitory effects at various levels of the mammalian olfactory system. Behavioral studies have shown that cholinergic modulation is involved in olfactory short term memory in the olfactory bulb, as well as in olfactory learning and memory. Physiological experiments show the effect of cholinergic modulation on synaptic transmission in the olfactory bulb (Elaaraghy et al., 1991). We have investigated biophysical simulations of the OB (Linster and Hasselmo, submitted) and the PC, both exhibiting realistic population dynamics in response to stimulus. The anterior limb (PFC) oscillations around 50-60 Hz in the OB as well as slow (3-8 Hz) and rapid (50-60 Hz) PP oscillations in the PC) and malacic single neuron responses (spontaneously and in response to olfactory input) (see Figure).

These models now allow us to investigate (i) the dynamic interactions between OB output neurons and the feedback from PC onto OB interneurons, (ii) the synchronization of neural spike trains synapsing in common pyramidal cells, (iii) the effect of neuromodulator in both layers and (iv) synaptic changes related to odor processing.


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Primary gustatory cortex in the macaque is located in frontal operculum (FO) and adjoining anterior insula (AI). Here we report on the characteristics of taste-responsive neurons in this area. Tastecells contained 5% of the neurons whose response properties were investigated. They occupied an area extending 4 mm posteriorly from the junction of orbitofrontal cortex with the FO, 3 mm mediolaterally, and 7 mm in the dorsoventral plane. Most action potentials were 1-200-500 Hz. The mean spontaneous rate was 3.4-4.9 spikes/sec. There was no indication of chemosensory characteristics in the gustatory cortex. Of 364 taste-responsive neurons, 43% was the most effective of the basic stimuli for 39% (n = 104), NaCl (n = 36), Nac (n = 207), quinine (n = 15) and HC1 for 13% (n = 49). Neurons most responsive to NaCl and NaC were more narrowly tuned (<0.01) than those sensitive to quinine and HC1. Across all stimuli, the ratio of excitatory to inhibitory responses was about 10:1. The mean response across all cells rose monotonically with increasing stimulus intensity, but the slopes of intensity-response functions were lower than those from human psychophysical studies. When these were calculated exclusively from the responses of cells in the appropriate subgroup, however, they matched intensity-response functions from human. Thus, perception of the four basic taste stimuli is probably carried by the subgroup of neurons most sensitive to a particular quality, rather than by the responses of all cells. An analysis of patterns of activity evoked by a wide range of stimuli indicated that taste quality coding in the macaque is quite similar to that in humans.

Supported by a research grant from the NSF.

686.8 FLAVOR AND THE FRONTAL CORTEX. B. Schut1, Y. Dodd1, and B.M. Stein2.

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Several lines of evidence indicate that the frontal cortex may be involved in the integration of odor and taste, the interactional combination of which produces the perception of flavor. To assess this, rats were first trained using operant conditioning to respond at a high rate when presented a solution of NaCl and arachidonic acid and to inhibit responding when only the odor or only the taste component was delivered. Concentrations of the stimuli were varied so that intensity could not be used as a discriminative cue. The maximum concentration of arachidonic acid was below gustatory threshold as determined in tests with normal and olfactory bulbectomized rats. After training, experimental (n=4) received a unilateral olfactory bulbectomy, transection of the anterior lobe of the anterior commissure and removal of the contralateral frontal cortex. Thus, these rats had no olfactory input to one hemispheres and no gustatory input to cortex in the contralateral hemisphere. Control rats received no surgery (n=5) or had all lesions in the same hemisphere (n=6). At controls showed good retention or rapid reacquisition of the flavor discrimination task and differences between operated and non-operated controls were not significant. In contrast, experimental rats had no retention of the task and showed no or only modest improvement in performance even after extensive retraining. In additional tests, all rats were able to discriminate the odor and taste component stimulus. These data suggest that the convergence of gustatory and olfactory input to frontal cortex is essential for the integration of taste and smell. (Supported by a grant from the US-Israel Binational Science Foundation, Jerusalem to Y.D. and S.M.S.)

686.10 TESTOSTERONE DIFFERENTIALLY REGULATES PHEROMONE INDUCED FOS EXPRESSION IN LDMIS and MEDIAN REAS OF MALE AND FEMALE Syrian Hamsters. L.M. Fibert* and J.M. Swan, Institute of Animal Behavior and Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

Exposure to female hamster vaginal secretions (FHS) specifically induces Fos in the posteroventral subdivision of the bed nucleus of the stria terminalis (BNTmP), the posterior subdivision of the medial nucleus of the amygdala (MeP), and the magnocellular subdivision of the medial preoptic nucleus (MPNmgm) of male hamsters. We have also previously reported a sex difference Fos expression in these regions after FHS exposure. In the present study we sought to determine the role of testosterone in regulating pheromone induced Fos expression in both males and females.

Adult male and female hamsters were gonadectomized and treated with a 2mm testosterone capsule (TC) or with an empty capsule (EC). Twelve weeks later, animals were exposed to FHS or water (no stimulus). Brain tissue was processed for Fos immunocytochemistry.

Our results indicate a sex difference in testosterone regulation of pheromone stimulated Fos expression in the BNTmP, MeP, and MPNmgm. Males (n = 5) show a greater number of Fos immunoreactive cells (IR) within the MPNmgm that females (+TC) (n = 5) females + EC (n = 5) males + EC (n = 5), and all control groups (n = 3 for each group) (p < 0.01). Both the BNTmP and MeP show FHS induced Fos expression in males + TC and + EC above that of controls and FHS exposed females + EC (p < 0.01). In FHS exposed females + EC, the BNTmP and the MeP are not stimulated to express Fos above controls, however testosterone acts in females to reduce this effect (p < 0.01). Thus, circulating testosterone influences pheromone stimulated Fos in the BNTmP and MeP of females, and in the MPNmgm of males. These differences in pheromone stimulated Fos IR implicate these brain regions in subserving testosterone regulated behavioral sex differences in response to exposure to FHS.

Supported by NICHHD R29-284647 and Rutgers University Research Council Grant to J.M.S. and a Sigma Xi award to JMF.

FORMATION AND SYNAPSY OF SUPRASYNAPSE S 1

687.1 MORPHOGENESIS OF AN IDENTIFIED MOTONEURON IN RESPONSE TO TARGET MANIPULATION. J.V. Fernandez and H. Keshishian*. Biology Dept, Yale University, New Haven, Connecticut.

We are examining nerve-muscle interactions during the development of the adult Dorsal Longitudinal flight Muscles (DLMs) of Drosophila. The fibers develop during metamorphosis from three persistent larval muscles, and are innervated by 5 motoneurons. The wing, dorsal and larval DLMs are innervated by a single motoneuron, MNS. Using intracellular Lucifer yellow dye fills, we examined the target dependence of MNS's development during metamorphosis (from 18-38p APF; after puparium formation). The peripheral synaptic arbors is confined to muscle fibers and 1 throughout the stages examined, and corresponds to the entire component previously characterized by anterograde labeling. In the CNS, the dendritic arbor arises about 10-12h after the differentiation of the peripheral motor endings, indicating that they do not develop in synchrony. Dendrites appear at 25p APF, at a time when the peripheral arborization on the muscle fibers is well elaborated. We next examined the effects of muscle fiber deprivation/inhibition on MNS. Following ablation of the larval template for DLM e and f, adult fibers develop de novo, although in a delayed fashion. Interruption to these fibers is corresponding delayed (Fernandez et al, Soc. Neurosci. Abst. '94). In the CNS we did not observe any effect on the dendritic arbors during stages 26-30p APF. We are further examining CNS arbor development at earlier stages to determine whether this neuronal compartment is adapted in response to peripheral delays. Target deprivations lead to ectopic muscle endings made onto the remaining fibers, as observed using anti-HRP. Our dye fills shows that MNS can be the source of these future synaptic inputs. These results show that lining of MNS motor ending development is closely tied to the developmental stage of the innervated muscle fibers, and that this compartment of the neuron develops under local cues.

687.2 THE Drosophila mutation Passover alters gap junction function in the Giant Fiber pathway. Y. Sun* and R.J. Wyman.

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Mutations of the Drosophila gene Passover and of a C. elegans homolog, unc-7, alter specific electrical synapses in their respective organisms. The proteins mediate these genes (Cell 72:967-977; Genetics 133:527-541), and several other sequence similar genes, may have a similar function to the gap junction structural molecule, connexin (Trends in Genetics 10:303).

In Drosophila, an electrical synapse connects the Giant Fiber (GF) and the tegumental/motorneuron (TTMm) (J. Neurophysiol. 64:405; J. Neurocytol. 2:753). In flies mutant for the GF which are also GF deficient, the synapse becomes abnormal. In this report, we show that the Giant Fiber also makes electrical synapses with a commissural tract in the brain. In Pass flies, the synapses connecting the Giant Fiber to the TTMm and the commissure are either absent or malfunctioning.

We injected cobalt and HRP into the TTMm muscle and into the mesothorax. The giant fibers ascend to the brain from the cervical connective and then fan out in a dorsolateral trajectory. In wild type, but in Pass, cobalt passes from the first six gap junctions into several fibers of a commissure just dorsal to the esophagus. The commissure fibers connect the two GFs to each other. HRP, which does not pass gap junctions, does not pass into the mesothorax fibers. Cobalt also passes from the TTMm to the GF in wild-type, but not in Pass. We conclude that the gap junctions in these two electric synapse are malfunctioning in Pass. Supported by NIH NS-07314; NSF IBN-9213387.

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During development and adult reinnervation, transient multiple innervation of neuromuscular junctions is followed by a period of synaptic elimination which continues until reinnervation. We are interested in understanding the modulation of this process by activity. Synchronous activity among the afferents, in vitro, is perhaps mediated by transient electrical coupling among motor neurons, may be important for the establishment and transient maintenance of multiple innervation, while asynchronous activity among the efferents may lead to synaptic elimination. To begin to evaluate this hypothesis, we used immunohistochemistry to show that before and at both stages of the motor neurones in the rostral lumbar sympathetic cord express the gap junction protein cx32 (antibody provided by Dr. D. Paul). Immunohistochemical staining showed that motor neurones were electrophysiologically connected during this period. Gal lent in vivo (Gal and Walton, 1996). The decrease of motor neurones expressing cx32 declined after birth until p14, as in adults, if any positive motor neurones were detected. Similar results were obtained from the sternomastoid motor pool. Given that multiple innervation is transiently recapitulated during adult reinnervation, we used cx32 immunohistochemistry to determine whether gap junction proteins are re-expressed in adult motor neurones following double nerve crush. One week after crush, when multiple innervation is widespread, ca 70% of motor neurones ipsilateral to the crush stained positively. Two weeks after crush, when synaptic elimination is underway, 50% of motor neurones were positive; four weeks after crush, when single innervation is re-established, less than 10% of motor neurones were positive. In both development and adult reinnervation, cx32 expression in motor neurones was the most widespread during the time multiple innervation is present, and both disappeared with a similar time course. We are using physiological methods to characterize the extent of electrotonic coupling and the synchrony of activity among motor neurones during development and reinnervation.


Evidence derived mainly from in vitro experiments has led to the hypothesis that agrin released from motor axon terminals directly initiates the events of postsynaptic apparatus formation in neuromuscular junctions by inducing myoblasts to make local aggregates of AChRs and AChE and certain other proteins. Determining by in vitro studies whether both agrin in an earlier event such as the induction of junctional folds and the accumulation of myosin filaments is difficult by the low survival rate and uncertain behavior of myoblasts maintained for prolonged periods in culture medium. We have performed an experiment that permitted a detailed analysis of neuronal agrin's role in all aspects of postsynaptic apparatus formation is the denervated adult rat soleus muscle, in which myoblasts are competent to form postsynaptic apparatus throughout much of their length. We injected DNA coding for agrin neuronal into the extrajunctional re-gions of such muscles. The coding included a rabbit non-repetitive sequence to direct the protein into the myoblast's secretory pathway. 7X, muscle fibers expressing n-agarin and those adjacent to them had on their surface co-localized aggregates of AChR, AChE and several other postsynaptic proteins as detected by immunofluorescence microscopy. By eye, the AChR/AChE aggregates induced by n-agarin were accompanied by a preferential localization of myosin and the presence of folds in the plasma membrane similar in size and shape to junctional folds, as detected by electron microscopy. These results make it likely that the interaction of agrin-released n-agrin with agrin receptors on myoblasts at the developing neuromuscular junction induces at least some late as well as early events in postsynaptic apparatus formation. Further experiments with transfected soleus muscles may provide insights into whether n-agrin plays a role in the induction of other late events such as changes in the gene regulation of AChR subunits.


Cranin was described in 1987 as a membrane glycopeptide expressed in brain and many other tissues, which binds laminin with high affinity in a calcium-dependent manner. Dystrophin-associated glycoprotein (dystroglycan) is a laminin-binding protein cloned in 1992 whose role in cranin remained uncertain. Using protein purified from homogenate from sheep brain, we now show that cranin is a form of dystroglycan, based on amino acid sequencing of 120 KDa and 43 KDa bands, and on carbohydrate cross-reactivity with antibodies directed against synthetic peptides derived from dystroglycan sequences. These data also localize the N-terminus of B-dystroglycan to amino acid residue 654. We find that cranin is a neuronal-specific protein which is tightly associated with membranes, and localizes to regions of CNS synaptic contact as assessed by immunocytochemistry of rat cerebrum. Brain alpha-dystroglycan is present at developing cerebellar granule cells, terminal GallNac residues, and the HKN-1 epitope. Though dystroglycan has been presumed to be a proteoglycan, the amino acid sequence in this brain alpha-dystroglycan is highly homologous to the carbohydrate-binding sites of the carbohydrate-binding domains of the mammalian a1-antitrypsin, lectin-binding and laminin-binding properties of brain dystroglycan are more typical of mucin-like proteins. Furthermore, using CHO cell lines deficient in xylosyltransferase and galactosyltransferase I, which are required for glycosaminoglycan biosynthesis, it is shown that chondroitin sulfate and heparan sulfate are not critical for laminin binding, and indeed are apparently not expressed in dystroglycan from CHO cells. Supported by NIH HD 09402, NS 26055 and the Brain Research Foundation, Inc.

IN VIVO EXAMINATION OF ACETYLCHOLINE RECEPTOR TURNOVER IN DENERVATED NEUROMUSCULAR JUNCTIONS USING CALIBRATED FLUORESCENCE IMAGING. E. L. Miller, M. S. Duguid, Department of Anatomy and Neurobiology, Washington University School of Medicine, St.Louis, MO 63110.

Using a quantitative imaging technique to follow changes in the absolute intensity of a fluorescent marker, we can examine the turnover of acetylcholine receptors (AChRs) labelled with rhodamine conjugated alpha-bungarotoxin (R-ITX), an ion channel mediator, in situ in the developing mouse of living mice. This technique allowed measurement of the intensity of fluorescence in a defined junction, permitting assessment of changes in AChR density that were independent of changes in junction size. With this method we have found that at sites within innervated junctions the half-life of A ChRs is shorter than that within denervated junctions the half-life of AChRs is approximately 2-3 days.

ACHRs at denervated junctions are mixed populations; some receptors are the remnant of those present in the membrane before the denervation, while others have been inserted after denervation. Several studies have suggested that these two populations respond differently to denervation. To follow ACHRs that were in the membrane before denervation, we labelled them with R-ITX just before nerve cut. Conversely, to examine new receptors, we pre-labelled denervated junctions with unlabelled BTX three days after denervation, blocking all the receptors that were present in the membrane prior to denervation. The newly inserted ACHRs were labelled with R-ITX 7 days after denervation. For both populations, intensity measurements at individual junctional sites were begun ten days after denervation. At subsequent timepoints the junctional sites were relocated and re-imaged. Changes in the absolute intensity of the rhodamine label was found at various sites were each determined. Our results suggest that both populations of ACHRs are lost from the membrane at the same rate (t½ approx. = 2-3 days).


Since laminin and agrin compete for binding to muscle a-dystroglycan (Cell 77:675-686, 1994) the question arises whether laminin affects the distribution of surface molecules in the same way as agrin. When embryonic Xenopus myocytes were cultured without agrin or laminin, clusters of acetylcholine receptors (AChRs) had colonized laminin-binding sites (LBS) and phosphorytrosine (PY). The myocytes also exhibited a widespread distribution of microclusters of LBS which did not contain any detectable AChR or PY stain. When agrin (a gift from E.W. Godfrey and R.M. Nkiti) was included in the culture medium there was a large increase in the incidence of clusters containing ACHRs, LBS and PY, and not much decline in the density of LBS microclusters. By contrast, laminin caused a large decline in the density of the widespread LBS microclusters and a concomitant increase in the incidence of larger clusters of LBS. AChRs and PY were rarely detected at these laminin-induced clusters of LBS. The results suggest that (1) laminin has cluster-inducing activity which is different from that of agrin, (2) the clustering of LBS, unlike the clustering of AChRs, does not require tyrosine phosphorylated proteins, and (3) unclustered LBS on the cell surface are mobile, as previously suggested for unclustered a-dystroglycan (J. Cell Biol. 129: 1093-1101, 1995). (Supported by MRC of Canada)

IDENTIFICATION AND LOCALIZATION OF ARIA RECEPTORS IN SKELETAL MUSCLE AND MUSCLE CELL LINES O. Cortes, T.S. Khorst, C. Lee and G.D. Eichbach. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115 and *The Scripps Institute, La Jolla, CA 92037.

ARIA is a protein that promotes the synthesis of acetylcholine receptors in muscle, and thus may play a role in the development of the neuromuscular junction. ARIA is a member of a family of factors that are ligands for members of the EGF receptor (EGFR) family, i.e., HER2, HER3 and HER4. In order to determine which of the EGF receptor family members are involved in ARIA's action in skeletal muscle we studied the expression and localization of these proteins on skeletal muscle, and their ARIA-induced tyrosine phosphorylation on L6 cells. We have found that in L6 and 10T1/2 cultures ARIA is associated with directed against all these receptors are capable of precipitating a portion of the p185 signal. Antibodies raised to a peptide shared by all the members of the EGFR family precipitated all the p185 signal, which appears as a doublet. Anti-HER2 antibodies precipitated much a smaller portion of the tyrosine phosphorylated protein than antibodies directed against the other receptors. In all cases, the only tyrosine phosphorylated protein precipitated by these antibodies was p185. We have studied the expression of these receptors in skeletal muscle using immunocytochemistry with anti-receptor antibodies. HER2 and HER4 immunoreactivity appear to be concentrated at neuromuscular junctions. HER3 antibodies gave inconclusive results.

These results suggest that HER2, HER3 and HER4 may participate in the signalling of ARIA on muscle cells, and that at least some of these receptors may be concentrated at mature neuromuscular junctions.
687.9
NEUROTRANSPORT REGULATION OF ARIA EXPRESSION.
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ARIA, an astrocyte-receptor-inducing activity, initiates a cascade of
events leading to the expression of posttranslational components of the
neuromuscular synapse. These include the expression of ACHe and sodium channels. We now have evidence suggesting that
neurotrophins from the posttranslational muscle cell augment the expression of ARIA in the presynaptic neuron. Using embryonic rat ventral horn cultures containing motor neurons expressing ARIA, we have found that the muscle-derived neurotrophins NT-3 and BDNF increase the amount of ARIA mRNA by up to 3-fold. This effect peaks at 4 hours of treatment at a half maximal concentration of 10-100 pM. Under identical conditions, there is no change in messages for the low affinity receptor for NGF, or other presynaptic proteins. Furthermore, NGF, LIF, CNTF, or ARA do not alter ARIA mRNA expression in these cultures. BDNF and NT-3 also increase the amount of ARIA protein activity secreted into the media of these cultures. We have found no evidence for a reciprocal effect in that ARIA does not affect BDNF or NT-3 expression in embryonic cultured rat myotubes. Even though these cultures respond to ARIA by inducing the phosphorylation of an 185 KDa phosphoprotein, the levels of BDNF and NT-3 mRNA are constant after treatment for up to 48 hours with ARIA. Such interactions between the neurotrophins and ARIA could serve to reinforce specific developmental programs at synaptic contact sites.

687.11
SYNAPTGENESIS BY CELLS DERIVED FROM THE HUMAN NT2 CELL LINE. Cha-Min Tang, Michael Margulis, Rebecca Hartley, and Virginia M.-Y. Lee, Dept. of Neurology, U. of Maryland and Dept. of Pathology, U. of Pennsylvania.
NT2 cells are believed to be immortalized equivalent of neural progenitor cells in the developing human nervous system. Treatment with retinoic acid can induce NT2 cells to commit to terminal differentiation into neurons (NT2N). While NT2N cells under current in vitro culture conditions can express a sequence of neuronal markers that recapitulates the maturation steps in the developing nervous system, they do not express some late events in neuronal maturation (i.e. functional synapses). We examined the maturation of NT2N cells under different culture conditions. When NT2N cells were co-cultured with astrocytes patch clamp recordings from them revealed synaptic activity that were not previously seen. These synaptic currents exhibited the biophysical and pharmacological characteristics of glutamergic synaptic transmission. Synaptic currents suggestive of GABA_B-mediated synaptic signals were also observed. These electrophysiological data were supported by positive immunohistochemical staining for synaptophysin, a synaptic vesicle protein. When NT2N cells were co-cultured with striate muscle they formed functional synapses that are reversibly blocked by curare. These findings suggest that NT2N cells have the capacity to express functional synapses and a variety of neurotransmitters.

687.12
PRESYNAPTIC INFLUENCE ON POSTSYNAPTIC CHANNEL KINETICS IN CULTURED SYMPATHETIC NEURONS. J.C. Thapar, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.
The nictinic channels of B- and C-cells, the principal neurons in the bullfrog lumbar sympathetic ganglia, have different kinetics (J. Neurosci. 6:590, 1986). B- and C-cells are intermixed selectively by B- or C-preganglionic neurons, which are located in separate regions of the adult spinal cord (J. Comp. Neurol. 268:71, 1988). This study examined the influence of presynaptic inputs on postsynaptic channels by comparing synaptic currents of B- and C-cells that had been co-cultured with spinal cord segments that contained either B- or C-preganglionic neurons. Miniature excitory post synaptic currents (mEPSCs), evoked by focally applied sucrose, were recorded from ganglion cells using the tight seal whole cell voltage clamp technique. The mean (+SEM) decay rates of mEPSCs recorded from ganglion cells co-cultured with B- or C-preganglionic neurons were 6.08 ± 0.25 (n=42) and 8.56 ± 0.43 (n=27) ms, respectively (p=0.001). These results suggest that the kinetics of synaptic channels expressed in cultured sympathetic neurons are influenced by the type of innervating preganglionic axon. Future work will examine the influence of presynaptic axons on channels expressed by ganglion cells from early stage tadpoles.
688.1 PROSTAGLANDIN INACTIVATION IN THE BLOOD-BRAIN BARRIER: ROLE OF THE CHORDOID PLEXUS, N. Krunic, J. Birdial, L. Albers, J. Price, J.K. Opolony, Department of Neuropathology, Toronto, ON, Canada M5G 1X8. (Samuel Lufness Res. Inst., St. Michael’s Hospital, Toronto, ON, Canada M5G 1X8).

In early gestation, prostaglandins (PGs) are most likely inactivated by local catabolic enzymes. This enzyme activity disappears by birth and, in adults, PGs are actively transported across the blood-brain barrier and chordoid plexus for catabolism in the periphery. We investigated whether PG transport across the plexus and brain increases at birth in association with the marked decrease in central PGE2 levels. PG uptake in the chordoid plexus from fetal (13-16 d; term 145 d), newborn (3 d), and adult sheep was studied in vivo using [(14C)]prostaglandin as substrate and [(14C)]acetic acid as extracellular marker. Fetal chordoid plexus accumulated PGF2a, reaching levels of 3.0-4.0 pmol/mg tissue with a t1/2 of 21 min and a mean extraction ratio (T/M) of 5.3 ± 0.5 (n=8). Newborn results were similar (T/M=7.0 ± 1.4, n=8). Uptake in the adult reached steady-state at 60-90 min (T/M=13.6 ± 1.3, n=8) and was significantly higher than fetus and newborn (p<0.05). Extracellular volume was constant at all ages (T/M=4.4 ± 0.02, n=23). Catabolism of PGF2a to its inactive 13,14-dihydro-15-keto (15KD) metabolite was analysed by thin-layer chromatography. Catabolism (radioactivity of 15KD expressed as %total) decreased significantly with age from fetus (52 ± 4%, n=8), to newborn (33±2%, n=7) to adult (no catabolism). PGF2a uptake by the fetal chordoid plexus had a high capacity (saturation at 40-60 pm) and was significantly inhibited by probenecid (1 mM, n=0). We conclude that the PG transport system of the chordoid plexus develops in utero in sheep. PG uptake in vitro is lower, whereas catabolism is higher, in the perinatal period than in the adult. There is no abrupt increase in PG uptake at birth, which implies this mechanism is not responsible for the rapid decrease in central PGE2 at this time. (NK supported by SIDS and Genesis).

688.3 HIGH GLUT1 GLUCOSE TRANSPORTER EXPRESSION IN HEMANGIOBLASTOMA ENDOTHELIA. E. M. Corford*, S. Hyman, K. L. Black, M. E. Corford, H. V. Vinters and W. M. Paradis. UCLA School of Medicine, Los Angeles, CA 90024; West Los Angeles V A. Medical Center, Los Angeles, CA 90073; and Harbor-UCLA Medical Center, Los Angeles, CA 90059.

Light microscopic immunocytochemistry indicated the enriched presence of GLUT1 glucose transporter throughout the central endothelia in a resected hemangioblastoma. Glibellar fibroblastic acidic protein (GAPF) was observed only at the tumor border; no GAPF-reactivity was seen in stromal cells, pericytes or endothelia in central tumor regions. Quantitative EM immunogold analyses of GLUT1-positive sites per umeter of capillary membrane confirmed the GLUT1 transporter was highly enriched in tumor endothelial cell membranes. Immunogold EM using albumin (MW 65000) indicated that HSA moved freely from the vascular lumen into pericapillary regions, confirming the "leaky" barrier (seen in clinical neuro-imaging). GLUT1 is generally said to be more concentrated in erythrocytes than in any other cell type in the body. GLUT1 in these tumor capillaries was more than 2-3-fold higher than seen in normal human red cells. We conclude that in the absence of GAPF, a high GLUT1 density characterizes this tumor. The increased immunoreactive GLUT1 seen suggests this may be a useful model system for studying BBB GLUT1 regulation; some tumor-derived factor may induce GLUT1 expression in the hemangioblastoma endothelium. Supported by NIH grant NS 25554.

688.4 TRANSLATIONAL ACTIVATORS IN 5'- AND 3'-UNTRANSLATED REGIONS OF GLUT1 GLUCOSE TRANSPORTER mRNA. R. Baude and W. Piekarski. Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Recent studies have indicated that the blood-brain barrier GLUT1 glucose transporter is under post-transcriptional regulation. To undertake the functional mapping of the GLUT1 transcript, the present investigation studied the translational efficiency of full-length synthetic human (h) or GLUT1 mRNA, both 5'- and 3'-untranslated regions (UTR) deleted mRNAs. Translation of 10 ng full-length hGLUT1 capped mRNA in rabbit reticulocyte lysate produced 0.16 ± 0.01% of total TCA precipitable material (mean ± S.E., n = 3) after 30 minutes incubation at 30°C. Deletion of 5'-UTR and 3'-UTR completely blocked the translation of these GLUT1 transcripts. The putative role of these GLUT1 UTR cis-acting elements was studied using the luciferase expression vector pGL2. DNA corresponding to the hGLUT1 5'-UTR generated by PCR was subcloned at the HindIII site of pGL2 located upstream of the luciferase 5'-UTR. Transient transfections of cells with pGL2 containing the complete [nucleotides (nt) 1-171] hGLUT1 5'-UTR markedly increased the expression of luciferase (60.7 ± 1.43 vs. 10.2 ± 0.7 pg luciferase/mg, respectively). Insertion of hGLUT1 5'-UTR in reverse orientation induced no changes in the expression of luciferase (9.1 ± 1.7 pg/mg), and the insertion of an unnatural 171 nt decreased its expression (insertion of nt 96-171 of the hGLUT1 5'-UTR retained most of the stimulatory effect (41.3 ± 10.0 pg/mg). In parallel experiments, at 2100-2300 of the bovine (b) GLUT1 3'-UTR were inserted at the PM1 site of pGL2, which is located downstream of the luciferase 3'-UTR. This region of the hGLUT1 3'-UTR encompasses the binding domain of a 88 kDa Cys protein trans-acting factor. Transfection of C6 cells with this construct markedly increased (> 300%) the expression of luciferase. Conclusion: The present data provide evidence suggesting that the 5'- and 3'-UTR of the GLUT1 mRNA contains cis-acting elements involved in a translational activation of the GLUT1 gene in mammalian cells.


African sleeping sickness is thought to be due to a direct effect of Trypanosoma brucei gambiense and rhodesiense on anatomical structures in the vicinity of the third ventricle and, in later stages, on the entire brain. To cause disease, the parasite must first attach and migrate through the cerebrovascular endothelial layer. In this study we address the question of trypanosome adhesion to human brain microvascular endothelial cells by characterizing adhesion molecules that participate in this interaction. Parasites were passaged in mice and stained with echinulin bromide prior to incubation with confluent and confluent monolayers of human brain microvascular endothelial cells in 24-well dishes. (Golgi-lysosome-phycocerythrin FACs isolated). Attachment of parasites was quantified by fluorescence microscopy. Adhesion of parasites could be blocked by 65% in 4 hours by monoclonal antibody to E06-like antigen (1-70% by 48% by monoclonal antibodies to cellular adhesion molecule, CD 106 (VCAM-1)). Anti CD44 and anti ICAM-3 antibodies blocked the adhesion of parasites to endothelium. These findings suggest that attachment of trypanosomes to human brain endothelium is mediated at least in part by cellular adhesion molecules, including VCAM-1 and ICAM-3.

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BLOOD-BRAIN BARRIER: OTHER WEDNESDAY
**688.7**

CATIONIZED HUMAN IMMUNOGLOBULINS AS A TREATMENT FOR CEREBRAL ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS): EVALUATION OF PHARMACOKINETICS, EFFICACY, AND TOXICITY IN SCID-hu MICE. W.M. Parodi*1, J. Yang, J. Ruscik, and Y.-S. Kang. Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The treatment of central AIDs requires that drugs undergo transport through the brain capillary endothelial wall, which makes up the blood-brain barrier (BBB) in vivo. Antibodies are potential AIDS therapeutics and cationized antibodies undergo transcytosis via transferrin-receptor-mediated transcytosis (Trans. Natl. Acad. Sci. 86:4761, 1989). Cationized human immunoglobulins have inhibitory effects on the replication of the human immunodeficiency virus (HIV) in human lymphocytes in tissue culture (Lull, J. Exp. Med. 175:563, 1992). The in vivo efficacy of cationized human immunoglobulins in the SCID-hu mouse model was evaluated. Immunoglobulins from non-infected humans and from HIV-infected individuals were cationized. A pharmacokinetic analysis showed that cationized immunoglobulins were taken up into the intracellular compartment of human lymphocytes. Treatment of HIV-infected SCID mice that were transplanted with human lymphocytes demonstrated therapeutic efficacy at a dose of 5 mg/kg. Immunohistochemistry showed that immunoglobulins were localized to the intracellular compartment of human lymphocytes. The results of this study provide evidence that cationized human immunoglobulins are suitable candidates for the treatment of viral diseases, such as AIDS; cationized antibodies undergo enhanced transport into brain and peripheral organs; when homologous cationized immunoglobulins are administered, there is no measurable tissue toxicity.

**688.8**


A hallmark in the pathogenesis of central nervous system (CNS) inflammatory diseases is the presence of peripheral blood mononuclear cells (PBMC) in the CNS parenchyma. The results and mechanisms contributing to extravasation of PBMC are as yet to be completely defined. One molecule suggested to have a role in this process is monocyte chemotactic protein-1 (MCP-1). We have investigated the potential role of this molecule in transmigration of PBMC, we used a model of the human blood-brain barrier (BBB) that is constructed with autologous human fetal endothelial cells and astrocytes. freshly isolated human PBMC were labeled with rhodamine-conjugated dextran (DiI) and placed onto the endothelial (eg: luminal) surface of the model. MCP-1 (100 ng/ml) was infused into the medium surrounding the astrocyte (eg: abluminal) side. MCP-1 induced transmigration of PBMC was noted after 60 minutes of incubation and was maximal by 150 minutes. The ratio of transmigrated cells in MCP-1 treated cultures as compared with untreated controls was 14.5±0.2 minutes of incubation. This study supports the hypothesis that MCP-1 is involved in transmigration of inflammatory cells across the BBB and into the CNS parenchyma. This mechanism may contribute to the development of neuroinflammation and the associated with AIDS.

(Supported in part by USPHS grants MH 74867 and MH 52794)

**689.1**

PHYSIOLOGICAL CORRELATES OF ANISOTROPY IN HORIZONTAL CONNECTIONS: LENGTH SUMMATION PROPERTIES OF NEURONS IN LAYERS 2 AND 3 OF TREE SHREW STRIATE CORTEX. W.JL Bosking and D. Fitzpatrick. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Axon collaterals of layer 2/3 neurons form an extensive system of horizontal connections in the tree shrew striate cortex (V1). These connections extend for several millimeters, linking neurons that have similar orientation preferences characterized by a major axis that is less than 20 degrees of visual space. Axon collaterals of most layer 2/3 neurons are distributed anisotropically, extending farther and giving rise to more than one branch along an axis in the receptive field that corresponds to their preferred orientation (Fitzpatrick et al., '93).

We investigated the functional consequences of this precise modular and topographic specificity through extracellular single unit recordings from isolated single units in layer 2/3. For each unit, the classical receptive field (CRF) was determined using a minimum stimulation technique. Five to eight repetitions were averaged for each length response curve. The resulting length response curves for isolated units in layer 2/3 were then plotted. The CRF of each cell was approximately 4 long. Some units (6/13) showed a progressive increase in response to stimuli as long as 40 deg. These data clearly indicate that the activity of cells in layer 2/3 of V1 can be modulated by visual stimulation outside the CRF. We also have evidence that in some cases, stimulation outside the CRF is sufficient to evoke responses from layer 2/3 neurons, independent of the stimulation of CRF. Supported by EY0621.

**689.2**


Given the same texture stimulation of the receptive field (RF), V1 neurons generally respond more vigorously when the pattern outside the RF differs from that inside the RF compared to the case where texture is of a homogeneous type across the display. We term this effect extra-RF modulation. Here we measured the delay of the onset of extra-RF modulation relative to the response latency of direct RF stimulation. Single and multi-unit recordings were made in area V1 of an awake, behaving monkey. As the monkey fixated a given V1 neuron was initially stimulated with a homogeneously textured display flashed on for 100 ms while background was static. The texture stimulation, the contrast outside the RF appeared to fall back in depth through stereoscopic disparity cues. In other trials, the homogeneously textured background was replaced by random mechanical noise. The pattern over the RF itself remained the same throughout the texture display period. Compared to the case of the static homogeneously textured display, V1 neurons responded more vigorously following the change in the extra-RF texture pattern (i.e., the change in texture outside the RF caused extra-RF modulation during static RF stimulation). The delay of the onset of this extra-RF modulation was 30 to 50 ms relative to the normal response latency of the cells under study, or 80 to 100 ms relative to the change in texture outside the RF. The contrast sensitivity of the neurons remained unchanged. We present some conditions for the onset of extra-RF modulation to be observed. This study suggests that extra-RF modulation arises from reciprocal activity within the cortex rather than from direct feed-forward visual input. The delay in extra-RF modulation (which is closely connected to synaptic delay) is related to the possibility that extra-RF modulation can reflect computationally complex analysis of visual input.

**689.3**

COOPERATIVE SELF-ORGANIZATION OF ORIENTATION MAPS AND LATERAL CONNECTIONS. Joseph Tourtellot and Risto Mikulikallio, Dept of Computer Sciences, Univ. of Texas, Austin, TX 78712.

Recent experiments show that the lateral connections in the primary visual cortex self-organize from cortical activity, and that their patterns closely follow receptive field properties as orientation preference. Through large-scale computer simulations, we show that a simple Hebbian weight assignment for the self-organized lateral connections, that is, along a linear axis of oriented receptive fields, orientation maps, and the patterns of lateral connections. The model consists of an array of retinal receptors connected to a cortical network. Neighboring neurons are linked both by excitatory and inhibitory lateral connections. Parameters such as axon length or the gain parameter were varied. At each input presentation to the network, there is an input activity through the aff erent connections, and the output is the activity of the output units, all connection weights are modified by the Hebbian rule. As training proceeds, the aff erent connections organize into orientated fields and orientation maps. The typical features of V1 maps and fragments develop in the orientation map. At the same time, the length of the lateral connections develop into the correlation between the patterns of lateral connections and the cortical activity, the principal feature distribution, is altered in the aff erent weights, and correlations between such features, shared in the lateral weights.

**689.4**


Several lines of evidence indicate that inputs from beyond the classical receptive field play an important role in the visual processing in the primary visual cortex. Cortical neurons, Multi-input visual stimuli, such as those utilizing m-sequences (Sutter, 1965) are advantageous to studying these interactions, because these stimuli identify inputs to the receptive field that would be subthreshold if stimulated in isolation. With this technique we (Kelts, Neuron, 1990) demonstrated that the population receptive fields of local field potentials are modulated by a gain control driven by nearby visual input. We now extend these studies to single-unit and multi-unit recordings in macaque V1. Lateral interactions of this type play a role in the CNS pathology. The signals from neural responses to contrast modulation of a 249-region stimulus (baseline condition) to responses to the same stimulus (i.e., line orientation), are recorded in the lateral weights, and correlations between such features, stored in the lateral weights.

In a linear zone. Elongated along the orientation axis.

At a photoreceptor center. Links to all orientations.

At a fracture. Elongated along two orientation axes.

In a linear zone. Elongated along the orientation axis.

At a photoreceptor center. Links to all orientations.

At a fracture. Elongated along two orientation axes.
689.8

To study the laminar and columnar distribution of macaque V1, we have used two different approaches. We have studied the distribution of macaque V1 and V2 by stimulating the cortex with high frequency electrical stimulation and by using a double-extraction approach. The first approach used is a double-extraction approach, which involves stimulating the cortex with high frequency electrical stimulation and by using a double-extraction approach.

We have studied the laminar and columnar distribution of macaque V1, V2, and V3 by stimulating the cortex with high frequency electrical stimulation and by using a double-extraction approach. The first approach used is a double-extraction approach, which involves stimulating the cortex with high frequency electrical stimulation and by using a double-extraction approach.

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689.11

LUMINANCE CONTRAST GAIN, SPATIAL AND TEMPORAL FREQUENCY TUNING IN CO BLOBS AND INTERBLOBS OF MAMMALIAN STRIATE CORTEX MEASURED WITH INTRINSIC OPTICAL METHODS: E. A. Evrard and E. Kaplan.
Laboratory of Biophysics, The Rockefeller University, N.Y., N.Y., 10021.

The contrast of macaque cortex contains regions of high metabolic activity, the cytochrome oxidase (CO) blobs. Single unit studies report that their physiological properties are distinct from those of the surrounding regions, but other studies report no such differences. To circumvent the limitations of single unit studies, we used intrinsic optical imaging to compare the response of CO blobs to luminance contrast (LC), the tuning for spatial frequency (SF) and temporal frequency with those found in the interblob regions.

Reflectance changes from layers 2/3 of the exposed visual cortex of anesthetized paralyzed monkeys can be imaged with a CCD camera in response to patterns which varied in luminance contrast, spatial frequency, or temporal frequency. The images were analyzed with extensions of the Karhunen-Loeve procedure (principal components analysis) and yielded spatial-temporal activity maps.

The contrast gain of the macaque cortex, measured optically, is similar to that of M and magnocellular neurons. The contrast gain of cat cortex is similar to that of single cortical neurons. We shall discuss the differences between the responses of pixels of blobs and interblobs, and show that optical imaging of intrinsic signals can be used to study the LC sensitivity, SF and TF tuning in spatially localized regions of cortex, such as the blobs.

Support: NIH ROI MH51065-01, EH 6058 and ONR N0014-91-1-2079.

689.13

THE ROLE OF NITRIC OXIDE IN MODULATING THE VISUAL RESPONSE OF NEURONS IN CAT STRIATE CORTEX. P. Karos, M. J. Friedlander.
Neurobiology Research Center and Dept of Physiology & Biophysics, Univ. of Alabama at Birmingham, 35294-0021.

Nitric oxide (NO) has been postulated to play a role in synaptic plasticity and development of the mammalian forebrain (Gulya et al., PNAS, 87:5457-5551, 1990; Bohme et al., Eur. J. Pharmacol, 199:379-381, 1991). We recently demonstrated that NMDA receptor activation leads to NO production that enhances release of glutamate in synapomorph preparations from the cerebral cortex (Montague et al., Science 263:973-977, 1994), and that NMDA receptor activation leads to potentiated synaptic transmission at excitatory synapses in cortical tissue preparations (Harrington et al., Soc. Neurosci. Abstr. 21, 1995). In the present study, we have explored the role of NO in modifying visual responses in anesthetized, paralyzed cats. Three to five barrel micropipettes attached to tungsten-in-glass recording electrodes were used to deliver (a) nitric oxide (NOS) inhibitors or (b) micropipettes containing LNAME or L-arginine (L-Arg). (c) Their inactive D-antagonists, and (d) the natural substrate for NOS, L-Arginine. Thus, the endogenous NO turnover was manipulated while evaluating neural responses to drifting visual stimuli of optimal and sub-optimal configurations. For 15 of 22 neurons, NO blockade (via application of 20-80 nA through the LNA or LAMMA barreled) reduced the visual response and spontaneous activity by 20-80%. In 5 neurons, NO blockade specifically facilitated the visual response by 40-100%. Equivalent ionophoretic currents through barrels containing D-forms of the NOS inhibitors were ineffective. These findings suggest that NO or its metabolites may play a role in signal amplification in the intact primary visual cortex.

Supported by NIH EY05116, HSFG RG-61093 and the Helen Keller Foundation.

690.1

VOLTAGE-DEPENDENT STATE TRANSITIONS OF SQUID NEURONAL CALCIUM CHANNELS. M.B. McFarlane and W.E. Gillis.
Departments of Molecular and Cellular Physiology and Biological Sciences, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

We have studied the gating of fast-deactivating (FD) Ca channels of squid giant fiber lobe (GFL) neurons using whole-cell voltage clamp. Prolonged depolarization causes the appearance of a second open state of FD Ca channels whose existence is obscured by a difference in the rate of channel closure (measured from tail currents) that is roughly 2-fold slower than from the normal open state (O_2). Entry into O_2 follows an exponential time course (t = 200 ms at +160mV). Neither E_CA nor F_CA amplitude of channels in O_2 nor the activation kinetics of E_CA are significantly different from channels in O_1. FD Ca channels open faster (O_1) closing kinetics within 50 ms at -80 mV (t = 9-16 ms). Since this recovery is possible at potentials where channels are likely to be closed, channels in O_2 appear to open and close along a pathway independent from (but similar to) that leading to O_2. Further evidence for the O_2 to O_1 transition is supported by the blocking effects of NiCl_2. Removing prepulse depolarization, the level of Ni block increases as entry into O_2 progresses. Recovery (O_1 to O_2) is observed at 0 mV in the presence of Ni, as the O_1-blocked level returns to that of O_1 (t = 14 ms). These slower tail current kinetics would be observed as an increase in mean open time at -80 mV on the microscopic (single channel) level. Such behavior at negative potentials could lead to an increase in calcium influx at the motor terminal following repetitive giant axon stimulation.

690.2

Biotechnology Laboratory, University of British Columbia, 237-6174 University Blvd. Vancouver, B.C. Canada V6T 1Z3.

Nickel ions have been reported to exhibit differential effects on voltage-gated calcium channels. To obtain a more precise characterization of nickel action we have investigated the effects of nickel on four major classes of cloned neuronal calcium channels (N, O, T and R) in Xenopus laevis oocytes. Nickel caused two major effects: 1) block of macroscopic currents and 2) shift in the current-voltage relation towards more depolarized potentials which was paralleled by a shift in the voltage dependence of activation. Replacement of Ba with Ca reduced both the degree of nickel block and the effect on activation for N, O and T channels, but increased the nickel block for R channel. The concentration of Ca where the effects of nickel were less affected was described by a simple model in which nickel binding to a saturable site resulted in altered gating behaviour. Both the affinity for block and the shift in current-voltage were reduced upon the removal of external Ca. Nickel caused two major effects: 1) block of macroscopic currents and 2) shift in the current-voltage relation towards more depolarized potentials which was paralleled by a shift in the voltage dependence of activation. Replacement of Ba with Ca reduced both the degree of nickel block and the effect on activation for N, O and T channels, but increased the nickel block for R channel. The concentration of Ca where the effects of nickel were less affected was described by a simple model in which nickel binding to a saturable site resulted in altered gating behaviour. Both the affinity for block and the shift in current-voltage were reduced upon the removal of external Ca.
POLYMINES INHIBIT VOLTAGE-GATED Ca2+ CHANNEL CURRENTS IN DORSAL ROOT GANGLION NEURONS AND IN OOCYTES EXPRESSING THE N-TYPE Ca2+ CHANNELalphalB.

G.W. Ganglieter, R.A. Grauf, and D.M. Rod,

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Endogenous polymines interact with a variety of ligand- and voltage-gated ion channels and may play a role as neurotransmitters or neuromodulators in the central nervous system (CNS). The effects of polymines on glutamate receptors have been studied in detail, less is known about the interaction of polymines with voltage-gated Ca2+ channels (Scott, Rf et al, TINS, 16:153-159, 1993). Using whole-cell and two-electrode voltage-clamp techniques, we studied the effects of endogenous and structurally-related synthetic polymines on Ca2+ channel currents in dorsal Root Ganglion (DRG) neurons and in oocytes expressing Class B (N-type) Ca2+ channels.

High concentrations (100 M - 10 mM) of the endogenous polymine spermine (SP) and spermidine (SD) reduced the peak amplitude of high voltage activated Ca2+ currents in DRG neurons, with SP being more potent than SD. The synthetic polymine sarcine inhibited DRG Ca2+ currents only at 10 mM. In preliminary experiments, SP (100 M - 10 mM), SD (1 and 3 mM) and long chain diamines (100 M) reduced the peak amplitudes of expressed Class B Ca2+ currents, while equivalent concentrations of putrescine, cadaverine and diethylamine did not affect peak current amplitudes.

These results suggest that high concentrations of released polymines may have a preynaptic action, by affecting voltage-gated Ca2+ channels, as well as a postsynaptic action on both voltage- and ligand-gated channels to affect neurotransmission in the CNS.

This work was supported in part by NS 16143 (RAG).

609.5 CONCERTED ACTIONS OF MULTIPLE CALCIUM CHANNELS IN EXCITATORY NEURONAL TRANSMISSION. D.B. Wheaton*, A. Ransdall, and W. Yenn, Department of Molecular and Cellular Physiology, Beckman Center B103, Stanford University School of Medicine, Stanford, CA 94305.

We have investigated the concerted actions of different Ca2+ channel subtypes in triggering neurotransmitter release at CA3-CA1 synapses of rat hippocampal slices. The K+ channel blockers, 4-aminoipyridine (4-AP), was used to modify the properties of voltage-dependent (VDV) in hippocampal area CA1, elicited by field stimulation of the Schaffer collateral/commissural fiber bundle. Intracellular recordings with sharp electrodes showed that 100 M 4-AP broadened the AP recorded in CA3 somata by slowing the repolarization phase. 4-AP caused a two-fold increase in the slope of the field excitatory post synaptic potential (EPSP) and eliminated the dependence of synaptic transmission on N-type Ca2+ channels. The latter effect was reversed by lowering extracellular Ca2+ concentration (4Ca2+). The effects of AP broadening on Ca2+ influx through various calcium channels were studied by application of mock APs to voltage-clamped, cultured cerebellar granule neurons. Slowing the repolarization phase of a mock AP increased current carried by N-, P/Q- and R-type channels to a similar extent. The sensitivity of the field EPSP to variations in 4Ca2+ was studied in the absence and presence of 4-AP. The form of this relationship indicated that as Ca2+ influx was progressively increased, increases in synaptic strength would eventually approach saturation. The relative impact of spike broadening is greatly increased by the selective inhibition of either N- or Q-type channels. This is of potential physiological importance because either type of channel is selectively down-modulated. Our findings are consistent with a simple scenario in which N- and Q-type channels both contribute Ca2+ influx to help trigger neurotransmitter release in individual synaptic boutons. The dependence on multiple types of Ca2+ channels is not absolute, however, because simply increasing AP duration increases Ca2+ influx through both kinds of Ca2+ channels, thereby lessening the dependence of synaptic transmission upon any one subtype.

609.7 COORDINATION OF EXPRESSION OF N-TYPE CALCIUM CHANNEL SUBUNITs THROUGHOUT DEVELOPMENT. M.M. McHenry*, T.D. Copeland*, T. Strisenga*, H. Glitschmann*, M. Dandona and C.M. Beggs*, Dept. of Physiology and Biophysics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, ABL-Basic Research Program, NCI Frederick Cancer R & D Center, Frederick, MD 21702; and Dept. of Molecular Pharmacology, University of Innsbruck, Innsbruck, Austria.

The omega-conotoxin GVIA sensitive voltage-dependent calcium channel (N-type VDC) is implicated in many cellular processes which include neurotransmitter release, neuronal migration, and synaptic vesicle docking. The N-type VDC is comprised of several non-identical subunits as indicated by both biochemical studies (McHenry, et al., 1991; Wichter, et al., 1993 Leveque, et al., 1994) and molecular cloning. While there is little question that the alpha subunit (alpha 2) of the N-type VDC is comprised of several subunits, it is not known how these subunits interact. In a recent study, the identity of the beta subunit associated with the alpha1B subunit remained in question. In a sample of 142 pyramidal neurons, 60% of the neurons had the T current, directly recorded under SEVC or recorded, in current clamp, as a calcium spike of 15-20 mV of amplitude activated from membrane potentials more negative than -70 mV. The neurons which had the T current were localized in the mid-deep layers of the cortex, at more than 400 M from the pial surface and were in a band of 200 M of thickness located just below layer 1. Neurons that did not have the T current were found in all layers of the cortex (except in layer II) slightly more frequently in superficial layers (between 200 and 600 M from the cortical surface). These data provide a direct demonstration that low threshold calcium current is expressed in pyramidal neurons located in particular layers of prefrontal cortex. Supported by Grant P929-0347 from the DGICYT (Spain).

609.8 LOCALIZATION OF PYRAMIDAL NEURONS THAT HAVE THE T-TYPE CALCIUM CURRENT IN GUINEA-PIG PREFRONTAL CORTEX. L. Gesi, J.Barrionuevo* and L. de la Mata Instituto de Neuroscience y Departamento de Fisiologia, Universidad de Alicante, 03080-Alicante, Spain.

One of the calcium currents present in cortical neurons is the low threshold calcium current or T current, which are involved in the generation of rhythmic activity and bursts of action potentials. We have studied the distribution of the pyramidal neurons that have this current in guinea-pig prefrontal cortex. The experiments were done in coronal slices of 300 M thick, that were incubated for 1h in the presence of TTX, 1 M and TTA, 10 M, the neuronal morphology was studied with intracellular staining with solutions of 2% blue-nigrosin, 40% of the neurons had the T current, directly recorded under SEVC or recorded, in current clamp, as a calcium spike of 15-20 mV of amplitude activated from membrane potentials more negative than -70 mV. The neurons which had T current were localized in the mid-deep layers of the cortex, at more than 400 M from the pial surface and were in a band of 200 M of thickness located just below layer 1. Neurons that did not have the T current were found in all layers of the cortex (except in layer II) slightly more frequently in superficial layers (between 200 and 600 M from the cortical surface). These data provide a direct demonstration that low threshold Calcium current is expressed in pyramidal neurons located in particular layers of prefrontal cortex. Supported by Grant P929-0347 from the DGICYT (Spain).
690.9 INOSITOL-PHOSPHATES MODULATE LIDIP BILAYER RECONSTITUTED CALCIUM CHANNEL ACTIVITY. B.D. Cheek*, M. Sugimoto and R. Linha, Dept. of Pharmacology, University of Massachusetts Medical School, 550 First Avenue, New York, NY 10016.

The effects of the phosphoinositides on P-type calcium channel activity were studied in cerebellar membrane vesicles and on isolated P-channel protein using the "tip-dip" bilayer technique. Cerebellar membranes were obtained from freshly dissected cerebellum. The membrane vesicle preparation was implemented in 400 mM sucrose. Electrical activity was measured in solutions containing 80 mM BaCl2, 10 mM HEPES, pH 7.4 in the bath; 120 mM CaCl2, 10 mM HEPES, pH 7.4 in the pipette.

P-channel protein was obtained using the polyclonal, 1-arginyl N,N'-bis-(3-aminophenyl)-1-hexadecyl-3-morpholinopropane-3-sulfonate (FMPS) (1:4) complex to Sepharose 4B CL via a 1-4-Butanediol Diglycidol ether linkage. This affinity gel allowed the extraction of a protein from bovine cerebellum homogenate. The electrical activity was measured in solutions containing: 80 mM BaCl2, 10 mM HEPES, pH 7.4 in the pipette; 120 mM CaCl2, 10 mM HEPES, pH 7.4 in the bath.

To study the effect of phosphoinositides on electrical activity from membrane vesicles, the compounds were added to the solution in the pipette (cytoplasmic face of the channel) in concentrations ranging from 0.1 mM to 1.0 mM. IP3 at concentrations greater than 0.3 mM was found to increase the number of electrical events at any holding potential without an increase in the average open time per event. The electrical activity was blocked by the addition of STX to the bath. Neither FF nor IP4 produced an activation of the P-channel nor did the synthetic sterol steroid, cholesterol. IP3 was without effect when applied to the bath (extracellular face of the channel).

Studies of the phosphoinositides on the isolated, reconstituted P-channel produced an identical pattern of results. The activation of the P-channel was prevented by the addition of heparin at a concentration of 100 µg/ml.

Support: NIH-NS13742 and NIA AG09480.

691.1 DOPAMINERGIC RESPONSES TO STRIATAL INJURY. D.W. Howell*, G.T. Liberator, J.F. Wong, G.A. Deonna. University of Melbourne, Dept. of Medicine, Austin and Repatriation Medical Centre, Heidelberg, VIC 3084, Australia.

A potential therapy for Parkinson's disease lies in the stimulation and control of neurite outgrowth from nigrostriatal dopaminergic neurones. We have previously shown that the injury associated with implantation of gel foam to normal mouse striatum increases binding of [3H]-mazindol to preynaptic dopamine uptake sites 10 months after implantation (P<0.01). When combined with the observation by others of increased density of host tyrosine hydroxylase immunoreactive fibres after striatal grafting or cavitation, it seems likely that striatal injury is stimulating proliferation and sprouting of dopaminergic terminals. We have now shown that striatal cavitation induces a decreased response from the dopaminergic system with proliferation of striatal preynaptic dopamine uptake sites (P<0.05), increased tyrosine hydroxylase activity (P<0.05), and increased concentrations of dopamine (P<0.05), dihydroxyphenylacetic acid (P<0.05) and homovanillic acid (P<0.05). Interestingly, while the changes of preynaptic dopamine uptake site density are only seen ipsilateral to the striatal lesion, the changes in tyrosine hydroxylase activity, dihydroxyphenylacetic acid and homovanillic acid concentrations are bilateral. These preynaptic changes are accompanied by decreases of the predominantly postsynaptic binding of [3H]-SCH23390 and [3H]-spiperone to the D1 and D2 classes of dopamine receptors in the striatum. The density of preynaptic dopamine uptake sites, D1 and D2 receptors in the substantia nigra does not change after striatal injury. The striatal response to injury first becomes apparent 2 weeks after cavitation and gradually increases with time, resulting in a doubling of the DOPAC concentration and preynaptic dopamine uptake site density within 2 and 10 months respectively. If similar changes occur after intrastriatal transplantation in human Parkinsonism, the time frame and extent of the host dopaminergic responses would suggest that they may make a significant contribution to any observed clinical improvements.

691.2 XENOTRANSPLANTATION AND ANTIGEN MASKING OF FETAL PORCINE VENTRAL MESENCEPHALON IN A RAT MODEL OF PARKINSON'S DISEASE. J. DeBalsi, A. McDonald, W.B. Drace*, D. Cibis*, D. Penney*. Neurology Research Laboratory, Deaconess Hospital, Belmont, MA 02178.

A model of Parkinson's disease was created using 6-OHDA lesions in rats. Fetal porcine mesencephalon was transplanted into the denervated striatum and evaluated for dopamine release. The model was selected because of the similar dopamine release pattern and the ease of implantation. All animals receiving fetal transplants showed a significant increase in dopamine uptake compared to controls. In addition, all animals showed a significant reduction in MPTP-induced dopaminergic substantia nigra activity. The results indicate that fetal xenografts may be useful in the treatment of Parkinson's disease.


Biochemical denervation of the striatal function of MPTP-induced striatal dopamine lesions in monkeys can be achieved only with noninvasive imaging techniques. With PET, individual subjects can be scanned serially at pre-MPTP, and throughout the period of disease onset at the extent and stability of the MPTP lesion over time. Unilateral intracranial MPTP administration allows for PET measures of ipsilateral (lesioned) striatum to be evaluated relative to the contralateral (unlesioned) striatum. This paradigm significantly reduces the variance associated with interanimal variability. For these studies, parametric imaging of the FDOPA uptake rate constant (Km) were generated by Putak analysis. ROI's drawn on each control were transferred onto its corresponding post-drug imaging set. Pre-MPTP scans in adult male vervet monkeys (Chenopithecus aethiops) (n = 8) showed right/left striatal K1 ratios between 0.95 and 1.05. For the MPTP studies, subjects (n = 5) received 15 mg/kg of MPTP, 30 min prior to PET scanning and at post-MPTP times. 4.5-7.5 and 9-11 months. FDOPA plasma metabolites peptides were similar to controls. For four of five subjects, striatal K1 ratios varied less than 5% between scans. Differences in subjects who were still lesioned right/left striatal K1 ratios were 0.10 and 0.28. One subject showed stable post-MPTP ratios up to 7 months (0.12) but then showed partial recovery at 9 months (0.29). These results indicate that individual variability-reaction to MPTP occurs and that stability assessment can be obtained with FDOPA-PET. These methods provide a baseline for accurate longitudinal assessment of post-intervention (e.g. fetal transplant) strategies in this Parkinsonian model.

691.4 EMBRYONIC DOPAMINE CELL IMPLANTS IN HUMANS WITH ADVANCED PARKINSON'S DISEASE: RESULTS FROM 7 MONTHS TO 7 YEARS AFTER TRANSPLANTATION IN 23 PATIENTS. C.R. Food, B.B. Boren, S.A. Schuck, M. LeRoy, C.F. O'Brien, L. Thompson, L.O. Ramge, F. Kaddis*, C. Mazzotta, G. Edelberg*, and A.A. Amart, Univ. of Colorado Sch. of Med., Denver, CO 80262; UCLA Sch. of Med., Los Angeles, CA 90024; North Shore University Hospital, Manhasset, NY 11030; and Emory Univ. Sch. of Med., Atlanta, GA 30322.

Embryonic dopamine cell transplants are an evolving therapy for patients with advanced Parkinson's disease. Since 1985, we have performed 23 transplants in patients. Two were unilateral in caudate and putamen and 21 were bilateral into putamen using up to 16 needle passes. Only tissue from embryos 7 to 8 weeks post-conception was used. Tissue from a single embryo was used in 6, from 2 embryos in 13, and 4 embryos in 4 patients. Six of the first 12 patients were immunosuppressed. Clinical change was monitored by UPDRS and ADL as well as frequent videotaping and computer testing in the home before and after daily five doses of drugs. PET scans were performed when available. Overall results showed 1) "On" and "off" performance improved three to twelve months after transplant. 2) Immunosuppression offered therapeutic benefit. 3) Transplant growth and clinical improvement progressed up to four years after transplant. 4) Drug doses were reduced in most patients by about 50%. 5) Reduced severity of both "on" and "off" phases and improvement in motor complications was seen. 6) Improvement in memory or other cognitive tests, and patients with memory disturbance prior to surgery were made worse for many months after surgery. 7) Complications included an intracerebral hemorrhage leading to death one month later, a cortical stroke remote from the transplant site in a patient with cerebrovascular disease, and a small postanemic bleed in one needle tract. 8) Surviving tyrosine hydroxylase positive cells were seen in the patient who died. Further evidence for transplant survival was seen on fluorodeoxy PET scans.

Since 1988, we have used closely spaced needle tracts to deposit embryonic dopaminergic cells for treatment of Parkinson's disease. This approach through bilateral cranial craniotomies at the apex of the skull over frontal cortex required up to 16 needle passes. We have estimated that the risk of serious hemorrhage was <1% and that neurological morbidity was <1%. The technique has been adopted by other groups performing neurotransplants. Our 17th patient had a catastrophic hemorrhage (after about 300 needle passes had been made into patients under this protocol). To avoid serious morbidity, we are now trying a new transcranial approach entering the forehead via twist drill holes. Four needle tracts are used to deposit 30 mm cores of tissue along the long axis of patient. Tissue from 4 embryos was used in 4 patients and 2 embryos in two patients. Six patients have undergone this procedure and are 8 to 18 months post surgery. Surgical time was cut from 2 to 2 hours. Hospitalization and postoperative headache were reduced. 3/6 patients were discharged the day after surgery. Results parallel those seen in the total population of 23 patients on whom we have performed transplants. For the first 3 patients, Activities of Daily Living Score fell from 23 to 10 in the "off" phase and 8 to 4 in the "on" phase by 7 to 9 months after surgery. Drug doses were reduced by up to 50%.


We have been using pcd mutant mice, a model of recessively inherited cerebellero-olivary atrophy, to study the structural integration of transplanted wild-type Purkinje cells into the disrupted cerebellar loop (Ann. Neurol. 20: 138, 1986; Anat. Embryol. 176: 145, 1987; Neurochem. Res. 17: 475, 1992). The aim of the present study was to determine the recovery of behavioral responses after bilateral grafting of E12 cerebellar granule cell (GCG) transplants into the deep cerebellar nuclei of the hosts, according to a protocol that emphasizes reconstruction of the missing cortico-cerebellar projection (Anat. Embryol. 185: 609, 1992). Motor coordination was assessed on a video monitored rat treadmil apparatus for 8 weeks. The shamt-operated group (n=6) stayed on the rotating drum for an average of 3.3 sec preoperatively and 3.0 sec postoperatively; pcd mice in the transplant-receiving group (n=6) were able to stay on the drum for 3.6 sec preoperatively and 13.5 sec postoperatively; the graft-induced 250% improvement was statistically significant (P<0.02).

Moreover, grafted mice were able to sustain the abdomen raised above the ground on their limbs during movement, contrasting to the typical lowered, widened stance of pcd mutant. The viability of transplanted Purkinje cells was verified with immunocytochemistry for calbindin-D28 and ChAT receptor subunits. An axonal invasion was supplied by donor Purkinje cells to the deep nuclear complex of the host. Further, most of the transplanted Purkinje cells had migrated to occupy cortical localizations, and displayed a correct orientation of their dendritic trees toward the pia. Our findings provide the first demonstration of motor enhancement in a model of inherited cerebellar ataxia due to intercellular grafting of fetal cerebellar neurons. (Supported in part by USPHS grant award R29 NS20285.)
692.1
CORTICAL CONNECTIONS FROM PHYSIOLOGICALLY DEFINED NUCLEI OF THE SOMATOSENSORY THALAMUS OF MACAQUE MONKEYS 1, L.K. Knoblauch*, J.S. Florence†, N. Jain, and J.H. Kaas2
1 University of California, Davis, CA; 2Vanderbilt University, Nashville, TN.

We examined the topographic organization of thalamic nuclei that project to somatosensory cortical fields in macaque monkeys. Electrotopographically identified representations in anterior parietal fields 3a, 3b (SI), 1 and 2 were injected with retrograde tracers, then at the time of sacrifice the somatosensory thalami was mapped using multiunit recording techniques. Tracers were placed either into representations of the same body part in different cortical fields or into different body part representations of the same cortical field. Results indicate that the ventral posterior nuclear, ventral posterior inferior nucleus, ventral posterior superior nucleus and ventral lateral nucleus contain separate complete representations of the body surface, and by different, expansion properties, stimulus preferences, architecture, and thalamocortical connections. Projections from the thalamus to electrophysiologically identified representations in cortex were both divergent and convergent, with projections to body part representations within a cortical field were not strictly homotopic. Such heterogeneous projections may provide the anatomical substrate for somatosensory cortical plasticity in adult primates after peripheral denervations.

692.2
HUMAN THALAMIC NEURONS RECEIVE SUBLIMINAL INPUT FROM REGIONS THAT ARE ADJACENT TO THEIR NATURAL MECHANICAL RECEPTIVE FIELDS. Z.H.T. Kim*, K.D. Davis, R.B. Tucker, A.M. Lozano, I.O. Dostrovsky. Departments of Surgery and Physiology, University of Toronto, Toronto, Ontario, Canada.

Acute deafferentation has been shown to cause rapid alterations in neuronal receptive fields (RFs) in the thalamus. The RFs of tactile neurons tend to shift/expand into regions immediately adjacent to that of the original RF, when input from the original RF is eliminated. The aim of this study was to test the hypothesis that thalamic neurons receive subliminal inputs from regions adjacent to their receptive fields, as such inputs might form the basis for the deafferentation-induced effects.

During stereotaxic thalamic exploration in patients being treated for pain or tremor, microelectrode recording identified neurons responsive to mechanical stimuli delivered to small RFs on the hand. In 10 patients, the electrical stimulation (ES; 1-2 μA, 0.5 ms pulse width, 1.0 Hz) delivered within and outside the RF was examined. Eleven of 23 cells were excited by ES within the RF and post-stimulus time histograms demonstrated a latency to activation of 22.3±2.5 ms (mean±SD). Of the 51 sites tested with ES outside the RF, 8 cells at 14 different sites responded with a latency of 40.8±14.5 ms. ES both within and outside the RFs was also found to produce inhibition 22-57 ms after the ES. Latency of activation of human Vc neurons from outside the RF is significantly longer (p<0.02) than latency from within the RF, suggesting mediation by indirect polysynaptic pathways. These data also constitute the first direct demonstration of inhibitory inputs to human Vc tactile neurons. The results suggest that subliminal inputs may be important in mediating thalamic and cortical plasticity.

[Supported by the Medical Research Council of Canada]

692.3
OCCUPATIONAL HAND CRAMPS GENESIS PARALLELED BY DEGRADATION OF REPRESENTATION IN THE SI CORTEX. N.R. Bush, M.J. Kaczmarek*, W.M. Jenkins Keck Center for Integrative Neuroscience University of California, San Francisco, CA 94122

Repetitive, sustained gripping under conditions of high force, vibration and/or end range motion is associated with a task specific loss of voluntary control referred to as occupational hand cramps or focal dyskinesia of the hand. The etiology of this disorder is poorly understood and treatment success is limited. The study was designed to measure behavioral and neurological consequences of repetitive, sustained gripping with a vibratory load. Adult and monkey were trained to sustain a hand grip with 80 gms of force and a 250 Hz vibration for 1.5 second. Monkeys performed several hundred repetitions/day. In time: 1) a tremor developed in grasping/relaxing the handpale; 2) the speed of task performance declined; 3) weakness in closing the hand emerged; 4) increased task errors were recorded; and 5) difficulties emerged in other hand retrieval tasks. Compared to the control side, the representation of the trained hand was significantly smaller; 6) the size of the cortical hand area was significantly reduced from normal; 2) receptive fields were on average, much larger than normal; 3) in the majority of cortical penetrations, neurons had multiple receptive fields extended across entire fingers or multiple digits; 4) in contrast to normal, neurons at many sampled sites had pachymetric-like receptive fields extending across the entire hand surface; 5) many large receptive fields extended over large sections of the body surface while having small hand surface areas; 6) receptive fields overlapped with each other over very long cutaneous distances; and 7) digit representations were geographically disorganized. We hypothesize that this profound, experience-driven change in the quality of differentiated sensory feedback must contribute to the loss of movement control that marks the emerging focal dyskinesia. Humans and monkey studies are now being directed toward determining how sensory learning could be implemented to re-differentiate cortical representations of all or of the glabrous hand surface.

[Research supported by NIH Grant NS-10441, HRM and UCSF REAC.]

692.4
DEPLETION OF BRAIN SEROTONIN DOES NOT IMPAIR PLASTICITY OF FUNCTIONAL REPRESENTATION OF VIBRISSEAE IN RAT BARREL CORTEX. M.Koperstok, K. Turini, B. Dziewiecki Dept of Neurophysiology, Nencki Institute, Warsaw, Poland

Serotonin influences development of somatosensory cortex in rat (Bennet-Clarke et al., 1994) and the blockade of 5HT1 receptors impairs developmental plasticity of the visual cortex (Carr and Singer, 1991). We examined the effects of 5HT depletion upon plasticity of cortical representation of a row of vibrissae (visualized with 2DG) evoked by neonatal lesions of vibrissal follicles in hooded rats. On day 14, the rats were pretreated with desipramine HCl and one hour later received injection of 5,7 DHT. In one group unilateral vibrissectomy sparing row C was also performed. Serotonin immunostaining at the age of six weeks revealed that injections in rats injected with 5,7 DHT 70-90% of serotonin cells in the mitral cell layer were reduced; 1) D-lyoxycyclas (2DG) mapping of the row C representation was done on remaining rats. The vibrissae were clipped on both sides with the exception of row C, the rats were injected with 2DG 10 days later; 2) the rats were stimulated. Autoradiograms of brain sections cut tangentially to the barrel field were processed with an image analyzer and the sections were stained with diaminobenzidine (DAB). Measurements of the size of barrels on the intact side showed that their linear dimensions in the 5HT depleted rats were 5-10% smaller than in untreated littermates. More than two-fold increase of the 2DG labeled cortical representation of row C was found in both control and 5HT depleted rats. This somatotopical reorganization resulted in large increase of cortical representation of the spared vibrissae, even to the extent of 3-4 times the size of the intact condition. Supported by KBN grant 0392/P2/94/06

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692.1
NEUROTRANSPRIMARY AND TRK RECEPTOR UPREGULATION IN REGIONS OF TARGETED NEOCORTICAL CELL DEATH COINCIDES WITH DIRECTED MIGRATION OF THALAMIC/THALAMOFUROCHORDAL PRECURSORS. Y. Z. Wang* and J. D. Macklis, Dept. of Neurology & Prog. in Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA 02115

Transplanted embryonic neocortical neurons and clonal, multipotent neural precursors undergo directed migration and differentiation that is sharply limited to regions of targeted cell death in mice, in which pyramidal neurons undergo apoptotic degeneration. To investigate the mechanisms of microenvironmental alteration underlying this directed migration and differentiation that occurs approximately 4-21 days after initiation of neuronal degeneration, we have examined expression of neurogenic factors and trk receptors in genetically defined regions undergoing targeted apoptosis. We have found, within the regions examined, first signs of 3a, an antibody to a subset of adult host neurons, or their progenitors, migrate into these grafts. Fetal (E15) hamster AH tissue was implanted into the third ventricle of immunosuppressed adult baboons. Between 6 and 8 weeks after implantation, Ki67 staining was performed in four brain sections (5 μm) was immunostained using a neuronal (NF) antibody (RMO 108, gift of Dr. V. neher) that recognizes rat and human neurons. In 6 of 9 recipients, we were able to identify and retrieve the rabbit highly with the host. In each of these animals, large (20-30 μm soma diameter) neurons of presumptive host origin were seen in association with host fibers. Sections from each graft contained between 3-15 host neurons; some cells located 250-300 μm from the graft-host boundary. The migration of adult neurons, or their precursors, into fetal grafts may represent a previously unsuspected aspect of potential plasticity in the adult brain. Whether this is dependent on the site in isolation of implantation is not known. Experiments are underway to determine whether this phenomenon is unique to xenografts, perhaps reflecting the absence of endogenous sensory signals, or a general feature of homografts as well. Supported by NIH NS38175 (MNL) and NS 24292 (RFS).
692.5


High resolution maps of cortical function organization in vivo have both scientific and clinical importance. Optical imaging of intrinsic signals is particularly suited for this purpose. We are reporting here the first application of this method to the study of the primate somatosensory cortex.

Using mechanical tactile stimulation, we mapped the somatosensory representation of the hand in Macaque monkey area SI. The stimuli consisted of air-pressure pulses applied directly to the skin on the hand simultaneously. The skin was displaced either by using small flexible transmitting membranes, or by fine, directed jets of air. We explored the effects of stimuli with different temporal properties, varying the number of pulses, pulse duration and inter-pulse intervals. In addition we looked at the effect of changing the air-pressure.

Using this stimulation method, we were able to see the cortical representation of each individual finger. In the maps we obtained, stimulation of each finger activated a narrow band running across the post-central gyrus. As expected, these stripes were ordered continuously along the gyrus. We also mapped responses to stimulation of the skin of the hand with moving stimuli. The optical maps were confirmed by extensive multi and single unit recordings. All maps remained very stable over many hours of recording. Thus, it should be possible to use this method to reliably map the digit representation.

By optimizing the stimulus parameters, we were able to obtain the functional maps extremely rapidly. Averaging the responses to a very small number (~4) of stimulations was sufficient to produce clear maps. Thus, maps of the five digits could be obtained in as little as five minutes. This rapidity allowed us to apply this method for mapping functional borders in humans undergoing neurosurgery. In a hospital study we managed to localize the central sulcus and map the border of the hand representation in the somatosensory cortex [Supported in part by the Israel Science Foundation administered by Israel Academy of Sciences and Humanities]

692.6


The inputs to the VPL thalamus from the dorsal column nuclei, and the projections from the thalamus to the somatosensory cortex, especially area 3b, show a high degree of somatotopic precision. Individual cells in VPL, however, show considerable divergence and overlap in this cortical projection (Rausell & Jones, J. Neurosci.: in press). We asked to what extent can this divergence maintain a map in area 3b in the face of diminishing numbers of VPL cells. Portions of VPL were electrolytically lesioned following physiological identification, and after two weeks, the representation area in 3b was electrophysiologically mapped. The lesions involved up to 80% of VPL. Behavioral deficits included lack of appreciation of light tactile and proprioceptive stimuli, mainly in the contralateral hand. Reconstructions of the multisite recorded cortical maps made after two weeks showed no apparent change in the somatotopic map of area 3b, even after the most extensive of the VPL lesions. Other cortical areas were not investigated.

The preservation of even a few rod like bundles of cells of similar somatotopy within VPL may account for the maintained cortical map. If a small portion of the rod remains, the divergent connections may be sufficient to maintain the cortical map. However, the observed behavioral deficits suggest that the lesioned monkeys had problems with perception of stimuli. Further to this, the multiple inputs into area 3b, from other thalamic nuclei, callosal connections, and pain pathways, may also contribute to the maintenance of the cortical map despite the lack of a primary input.

Supported by NIH Grant NS 21377.

692.7


The ventrobasal complex of the monkey thalamus (VB) sends axons to ipsilateral postcentral gyrus in an ordered fashion. Recent evidence indicates that these thalamocortical projections exhibit substantial divergence and overlap (Rausell and Jones, J. Neurosci.: in press). This study addresses the issue of the extent to which this divergence can maintain a normal pattern of gene expression for molecules involved in plasticity of area 3b maps in the presence of reduced VB input. Lesions of varying extent were made in parts of the VB representation defined by multisite mapping. After one or two weeks, the thalamus and somatosensory cortex were sectioned and hybridized with radioactive cDNA probes for glutamic acid decarboxylase (GAD), calmodulin and various protein kinase c (CAMKIIc) and Cam1, Cam2, and Cam3 subunits of the GABA<sub>B</sub> receptor subunit mRNAs, or stained immunocytochemically for GABA, GABA receptor subunits, CAMKIIc and parvalbumin. In general, the presence of a remaining portion of a VB representation was sufficient to maintain a normal pattern of gene expression, local changes only occurring in cortex with the largest lesions. Divergent connections, therefore, maintain sufficient activity to support a normal balance of inhibition and excitation in cortex.

Supported by NIH grant NS21377

692.8

A ROLE FOR ASTROCYTES IN CORTICAL REORGANIZATIONAL PLASTICITY? E. Crompton*, A. Minticchi and T.P. Parent*. Inst. Hum. Physiol., University of Ancona, 1-60131 Ancona, Italy, and Dept. of Neurology, Bowman Gray School of Medicine, Winston-Salem, NC 27115, USA.

What triggers the mechanisms that determine cortical reorganization is poorly understood. Since the expression of transmitters is regulated by activity, it is possible that changes in transmitter levels could be a triggering event. In this study, antibodies to glutamate (Glu) were used to study the effects of reduced afferent input on cortical excitatory neurons in adult monkeys subjected to somatosensory deprivation by cutting the three nerves to the hand.

In the cortical SC, DCN, VP thalamus and first somatic sensory cortex, Glu-immunoreactivity (Glu-ir) was similar to that described in normal animals; patterns with reduced or absent Glu-ir were never observed and no appreciable differences were noted between the lesioned and normal side. No changes were observed in sections probed for CO or immunoreactive astrocytes. During the post-mortem interval, sections contralateral to the nerve cut showed that most cortical fields had a normal pattern of Glu-ir (pattern a), some exhibited a reduction (pattern b), and that in the central portion of the upper bank of the central sulcus which corresponds to the hand representation of the second somatic sensory cortex (SII), Glu-ir had virtually disappeared (pattern c). Adjacent sections probed for CO or stained with thionin showed that in the regions corresponding to those characterized by pattern c, CO slightly decreased and astrocytic proliferation was observed. Cortical regions characterized by pattern c also revealed that in the hand representation of SII, small-sized, intensely stained cells with processes radiating in all directions, which proved to be astrocytes, displayed Glu-ir.

As in previous studies, our findings indicate that Glu-ir is regulated by afferent activity. Additionally, changes in astrocytic Glu levels in the reorganizing cortex suggest a role for neuron-astrocyte/astrocyte-neuron signaling in the reorganization process. Such regulation of Glu levels in astrocytes and neurons may act as trigger for the biochemical changes underlying the structural metamorphosis occurring during a slow phase of reorganizational plasticity in the cortical cortex.
693.1


Last year we reported that spatial attention isolates cells in macaque area V2 from the influence of nearby stimuli. In past viewing, the response to a single flashing bar within the receptive field is often suppressed by a second, nearby flashing bar. However, when attention is directed to the location of the first stimulus, the suppression caused by the second stimulus is filtered out, and the cell responds as though the second stimulus were absent.

Our present results show that a related attentional process is at work in macaque area V4. As in V2, we found that the response to a single flashing bar within the receptive field is typically suppressed by the presence of a nearby, synchronous flashing bar. As in V2, this suppression is filtered out when attention is directed to the first flashing bar.

We have also recorded V2 responses when the flashing bar is presented against a variety of large bit-mapped “real world” images. The real world images typically activated the cells in a sustained fashion, but also suppressed responses to the bar. Attending to the flashing bar filtered out some, but not all of the sensory interactions resulting from these more complex stimuli.

693.2

TWO-DIMENSIONAL MAPS OF SPATIAL ATTENTION EFFECTS IN MACAQUE AREA V4. C.E.Connor*, D.C. Prendergast, J.L. Guettard and D.C. Van Essen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have shown previously that responses in macaque area V4 depend on both the distance and direction of attention relative to the stimulus in the receptive field (Connor et al., Soc. Neurosci. Abstr. 19: 974 (1993); Connor et al., Soc. Neurosci. Abstr. 20: 1054 (1994)). Responses to a single stimulus are often modulated by attentional focus, and a majority of cells are tuned for direction. The optimal direction of attention (e.g., left, right, above, or below the stimulus in the receptive field) varies between cells. We designed a new experiment to map these directional attention effects in greater detail.

The monkey fixated a spot on a video monitor while a set of 12 ring-shaped stimuli was presented in a square array surrounding the classical receptive field of the cell under study. On a given trial the animal was required to attend continuously to just one of these rings and signal when it changed shape while the other responses were suppressed by flashing 3 bars of optimal orientation, width and color spanning the central 50% of the classical receptive field. A majority of the cells tested in area V4 showed a significant effect of attention position in this paradigm. Spatial tuning for attention position was typically broad, with most cells showing greater than 50% maximal responses across at least a quadrant of the mapped region. The results provide further evidence that area V4 processes information about the positional relationship between the stimulus driving the cell and the current focus of attention.

693.3

TIME COURSE OF ATTENTIONAL EFFECTS IN MACAQUE AREA V4. D.C. Prendergast, C.E. Connor, J.J. Gallant* and D.C. Van Essen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Recent experiments have shown that attentional effects on single cells in macaque area V4 include a shift in response strength towards the locus of attention and a change in overall response strength depending on the direction of attention (Connor et al., Soc. Neurosci. Abstr. 19: 974 (1993); Connor et al., Soc. Neurosci. Abstr. 20: 1054 (1994)). We designed a behavioral paradigm to assess the time course of these effects when attention is suddenly shifted. A macaque monkey was trained to attend to one stimulus (a ring) as part of a shape discrimination task, while a second, unattended stimulus (a bar) was used to measure attentional tuning profiles. The trial began with the simultaneous onset of the fixation point, a probe (bar) stimulus in one of five positions within the classical receptive field (CRF) and a field of rings positioned in the opposite CRF quadrant. After a 1 sec delay the attended ring was cued via a brief (100 msec) blink at one of four positions surrounding the CRF. The criterion for the animal's correct choice was a saccade towards the ring while, after a variable time interval, the ring became C-shaped.

Of 27 cells recorded thus far in one rhesus monkey, 10 showed a significant (p < 0.05) shift in cell activity 10-100 msec after the onset of the attended stimulus. The maximum time for the transient effect was 250 msec. The rhesis monkey was trained to make the attended saccade in the direction of the centered CRF and to release the stimulus after a 250 msec delay. Thus, we were able to distinguish the transient from the sustained attentional effect.

To determine the temporal profile of these effects, we analyzed the average responses in each group of cells. The shift effect began to develop at around 50-100 msec and peaked at about 200 msec following the attentional cue. In contrast, the directional effect developed more slowly, starting at about 200 msec and reaching a plateau 400-500 msec after the cue. These results suggest that the neural events underlying spatial shifts of attention occur rapidly, while information about the positional relationship between stimuli and attention evolves more slowly.

693.4

NEURAL CORRELATES OF TARGET DETECTION DURING VISUAL SEARCH IN AREA MT. Geoffrey T. Buracas* and Thomas D. Albright. VCL, The Salk Institute, P.O.Box 1800, San Diego, CA 92186.

Focal attention has been shown to play a central role in perception of complex objects. Visual search is a psychophysical paradigm traditionally used to explore the dynamics and mechanisms of focal visual attention. Using this paradigm we have previously shown that both humans and rhesus monkeys exhibit serial search for targets defined by a conjunction of color and direction of motion, i.e., search times depend linearly on the number of distractors in a search array (Barrasa and Albright, 1994, Soc.Neurosci.Abst., 20:1, p.318). This linear dependency is commonly accepted as evidence for dynamical and voluntary allocation of focal attention.

Using an identical search paradigm, we have now explored neurophysiological correlates of focal attention in rhesus monkeys. Specifically, we have recorded responses of directionally selective neurons in area MT of a monkey engaged in a visual search task. The search target was formed by a unique conjunction of color (red or green) and direction of motion (preferred or null direction for neurons under study) of a texture inside a circular aperture. The target for each trial was specified by a sample presented prior to the search array. The latter was composed of eight apertures defined by all possible combinations of selected color and direction. The monkey was required to saccade to the target upon detection, or to maintain central fixation on target-absent trials. Approximately 40% of MT neurons exhibited significant response modulation when the search target was positioned within the receptive field. This modulation was expressed as an enhanced (--30% of the predicted baseline) of the neuronal response prior (90-200 msec) to a saccade to the target. The time course of the neuronal response modulation thus parallels behavioral target detection. The sign of the modulation is consistent with the process of target selection and may reflect the engagement of an attentional mechanism.

Supported by McDonnell-Pew Center for Cognitive Neuroscience.

693.5


Visual activity of neurons in the monkey lateral intraparietal area (LIP) is influenced by the direction of attention and by eye movements. To understand better how these mechanisms operate, we investigated the effects of attention and intended eye movements on the firing rates of LIP neurons during visual stimulation. Single cell responses were recorded extracellularly in one rhesus monkey (Macaca mulatta) under three different conditions: (1) during a fixation task in which the monkey attended to a visual field stimulus and released a hand-held bar when the color of this stimulus changed at an unpredictable time, (2) during the delay period of a memory-guided saccade task (e.g., the presentation of a brief peripheral target and the beginning of the saccadic eye movement), and (3) during spontaneous gaze without fixation or saccade targets. Under all conditions, the visual excitability of the recorded neurons was assessed by presenting randomized sequences of test flashes (100 msec) at a rapid rate (100-250 msec intervals) during periods of stable eye position. This enabled the sampling of a large number of trials in the absence of one or more sources of variation in the visual field, and the subsequent generation of surface plots for a given cell's receptive field (RF). Most LIP neurons displayed graded responses with a maximum at the center of the RF, decreasing toward the monkey's attentional focus. The shape of excitability was quite restricted and had sharp borders. In other cells, the RF had a complex surface with both excitatory and inhibitory zones. The state and direction of visual attention had an effect on overall responsiveness but also influenced different characteristics of the RF structure such as sharpness of spatial tuning, and center of gravity. This indicates that the encoding of visual space by parietal visual neurons is strongly linked to the behavioral context.

693.6

ATTENTIONAL MODULATION OF DIRECTION-SELECTIVE RESPONSES IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE MONKEY. S. Trope*, J.H.R. Maunsell. Baylor College of Medicine, Houston, TX.

We recorded from over 50 direction-selective cells in the superior temporal sulcus of a macaque monkey performing a reaction-time task to investigate the role of attention in the processing of motion signals. The monkey was trained to attend a moving spot (the target) and ignore the position of one or more of the other spots (the distractors) while maintaining fixation on a stationary fixation cross. In the first experiment one spot moved through the receptive field (RF) in the preferred direction while another spot moved outside the RF. Even though retinal stimulation was identical in both trial types a large majority of the cells (~85%) responded more strongly when the animal was instructed to attend to the stimulus within the RF, compared to trials on which he was attending the stimulus outside the RF (median increase 30%). In the second experiment both spots moved through the RF, but in opposite directions (preferred and anti-preferred directions of motion). Almost all cells (~90%) responded more strongly when the monkey was instructed to attend to the stimulus moving in the preferred direction. Compared to trials in which he was attending the spot moving in the non-preferred direction (median increase 15%). This result cannot be accounted for by shrinking receptive fields since it held true even when the two dots passed by each other very closely.

Our results demonstrate that while moving attention away from the receptive field will reduce a neuron’s response to its preferred stimulus a much stronger effect can be observed when attention is switched off without changing the location of the receptive field. In that case the response of the neuron is strongly biased by the direction of motion of the attended stimulus even when the unattended stimulus is a more potent sensory stimulus. These findings are amongst the strongest exarantal modulations of neural signals found in visual cortex to date and suggest an important role for attention in selectively enhancing neural signals related to stimuli of interest.

Supported by NIH EY09911.
693.7 DISTINGUISHING CORTICAL AREAS THAT ARE SENSITIVE TO TASK AND STIMULUS VARIABLES WITH FMRI E.A. DeYoe*, P.W. Schmitt†, J. Neitz Dept. Coll. Bio., Med. Coll. Wisconsin, Milwaukee, WI (Email: deyoue@its.neww.edu)

**Purpose:** Functional MRI was used to study the effects of a) stimulus presentation rate on parietal cortical activation induced by a visual stimulus. **Methods:** During gradient-recalled, echo-planar FMRI, 3 subjects viewed a flickering checkerboard stimulus under two task conditions. Parietal sources were targeted by a task. Single stimulation presentations lasted 0.5 sec, and were repeated at 4 different rates (from 0.2 to 2 per second) in blocks (trials) of 24 seconds each. For each voxel, FMRI response amplitudes were extracted for the different presentation rates and curve-fit by linear regression. Histograms of regression line slope revealed two asymmetric peaks. Voxels whose response increased with presentation rate were classified as rate dependent while those with constant response were classified as rate independent. **Results:** Voxels in V1 and several extrastriate visual areas showed strong stimulus rate dependence in both task conditions. However, during passive viewing, stimuli had to be presented nearly twice as fast as during active viewing to achieve the same response magnitude in V1. Such differences were even larger for extrastriate visual areas. In frontal cortex, results were distinctly different. The response was rate independent during active viewing, while under passive viewing there was little or no response. **Conclusions:** Stimulus rate-dependence may provide a method for distinguishing areas of the brain serving task-related functions such as attention from those areas primarily concerned with processing of visual information. Supported by NIH grants EY10244, and Core Grant EY01031.

693.9 COMBINED FMRI, EEG AND MEG IMAGING OF VISUAL ATTENTION M. Hukalainen, SP Alfors2, HI Aroen3, AM Pylväni, JF Pajunen, RI Ilmoniemi3, WA Kemppainen4, A Korjonen2, AK Lie4, R Nissinen3, BR Rosengren, GV Simonen2, CCG Sanderst2, NW Sanderst2, E. Boisgontier1, CI Medical Research Unit, Univ. of Helsinki, Finland, 1Albert Einstein College of Medicine, New York, 2Radiology, Helsinki Univ. Central Hospital, Finland, 4University of Oslo, Norway, 5Biohall laboratory, Helsinki Univ. Central Hospital, Finland, 6Kungsholmens General Hospital and Dept. of Physiol., Stockholm, Sweden.

The activation of human cortex was compared in four subjects using MEG, EEG and FMRI in visual attention experiments related to that of Corbetta et al. (J. Neurosci. 1991). Event-related magnetic fields and electric potentials were compared to hemodynamic changes observed using functional MRI. The passive condition was to fixate on a cross and ignore the stimulus moving across the screen every 700 ms. In the color, orientation, and movement conditions, the subject saw the same stimuli, but the task was to attend to stimulus color, orientation, and movement direction, respectively. Movement-corrected FMRI data from each subject were registered and averaged in Talairach space. Multiple areas were differentially activated during the attended conditions, including extrastriate, parietal, dorsal lateral prefrontal and anterior cingulate cortices. Multiple and temporally overlapping sources were observed using MEG and EEG. In the passive condition, electromagnetic methods show activity in V1 and V2 areas, with latencies from 70 to 160 ms. Signal strength increases were observed across all modalities during the attended conditions, with attention to color giving the largest responses in this particular task. During the attend condition, FMRI, MEG and EEG suggest that the strength of these sources is modulated by attention.


Following unilateral damage of cortex at the tempo-ro-occipito-parietal (TOP) junction, humans fail to notice and respond to stimuli that appear in visual space contralateral to the lesion. In cases of profound neglect, subjects neither detect nor orient head or eyes to stimuli in a topographic fashion. This is often observed in patients with lesions of the superior colliculus. The cortical region in the cat is located in the posterior one-half of the middle suprasylvian sulcus at the junction of areas V4 and V7. The broad similarity of cooling-induced neglect of stimuli to neglect in humans supports the idea that the neglect observed in humans is a result of a similar neuroanatomic syndrome in the cat's brain. This study examined the effects of reversible lesions of the superior colliculus on the responses of the cats to visual stimuli. Lesions were made using a cautery probe, and the effect of the lesions on the animals' visual abilities was monitored. The results showed that the lesions had no effect on the animals' visual abilities.

693.11 BASIS FUNCTIONS AND HEMINEGLICET A. Pouget1 and S. Caplan1.

1Brain Research Institute, UCLA and 2Salk Institute, San Diego.

Cortical lesions in the parietal lobe of human produces a neurological syndrome called heminegliget, in which patients tend to ignore sensory stimuli in the hemispace contralateral to the lesion. Several studies attempted to determine the spatial frame of reference affected in visual neglect and found that it can be retinotopic, head-centered, body-centered, environment-centered, and in some cases, object-centered, in all in the same patient.

We recently developed a theory of spatial representations in the parietal cortex based on the responses of single parietal neurons and the computational nature of sensorimotor transformations. The model assumes that parietal neurons encode their sensory inputs with basis functions (BF), a type of receptive fields that can be used to generate nonlinear motor commands. One of the important property of BF map is that the position of an object can be represented in several frames of reference simultaneously. We simulated a lesions of BF maps and found that, as observed in heminegliget, the model predicts deficits affect multiple frames of reference, including object-centered. This model can account for recovery from neglect that occurs over weeks and transiently after callosal lesions. The model demonstrates that neglect can be explained without assuming the existence of explicit representations of cartesian space or object-centered representations. It relies instead on basis function maps biased for the contralateral side of space. This is, to our knowledge, the first account of heminegliget at the single cell level.

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694.1


A model of chronic central pain after spinal cord injury (SCI) was used to document the temporal development of mechanical allodynia using validated models of nociceptive behavior. The same animals tested behaviorally were used to determine if dorsal horn neurons were more excitable to peripheral stimulation as evidenced by increased responses to brush, press, pinch, von Frey hair stimulation following spinal cord hemisection at T13. Electrophysiological recordings of wide dynamic range (WDR) neurons in the dorsal horn from L3-L5 were made both contralateral (n=25) and ipsilateral (n=25) to the hemisection. These data were compared to control values (n=32). Thresholds were determined as spikes per second, before, during and after the application of these natural stimuli. The responses to brush, pinch and von Frey stimulation at 9.41mN were increased in the hemisected rats bilaterally and these increases were statistically significant compared to control values (p<0.005). These data support the development of central sensitization of WDR neurons and provide a mechanism for the observed behavioral changes in the development of mechanical allodynia after SCI. (Supported by NIH grants NS 11255, NS 09743 and the Kent Waltrip Foundation).

694.2


Handpaw injection of formalin produces acute (Phase 1) and persistent (Phase 2) nociceptive behaviors in the rat. This model has provided critical evidence supporting a central contribution of saturating hyperresponsitivity of spinal neurons to the expression of persistent pain. Here, we evaluated the contribution of ongoing peripheral nerve inputs to Phase 2 pain behavior in addition to pain behavior (flinching), we measured formalin-evoked increases in arterial pressure and heart rate via an indwelling arterial catheter. All three measures displayed both phases of the formalin response. The arterial pressure response was highly correlated with behavior, and was dependent on formalin concentration (0.625 - 5.0%), indicating that it was largely driven by nociceptive input. Animals obtained from Charles River exhibited slightly, but significantly, larger Phase 2 flinching and heart rate responses compared to those from Bantin & Kingman. Lightly-anesthetized (0.7% halothane) rats exhibited robust increases in blood pressure, indicating that cardiovascular responses did not merely reflect somatosensory-cardiovascular coupling. We next evaluated the contribution of ongoing peripheral nerve activity to the expression of the Phase 2 pressor, tachycardia, and flinching responses. We locally-anesthetized the ipsilateral or contralateral (control) paw with a hydrophilic lidocaine derivative, QX-314 (2%) after Phase 1, but before Phase 2 began. QX-314 blocked Phase 2 pressor, tachycardia and behavioral responses only when injected into the paw that received formalin (2.5% or 10%). We conclude that ongoing activity from peripheral nerves is required for the persistent pain evoked by formalin. Supported by NS14153, DA 08377 and NS 07265.

694.3


Opiate analogics may act in part by activation of receptors in PAG, which in turn activate spinal projecting neurons in brain stem regions such as NRM. In the present study, the relationship between delta and mu opioid receptors and PAG and NRM neurons was examined. Rats and one Rhesus macaque were deeply anesthetized and sacrificed by perfusion with 4% formaldehyde. Tissue sections were stained using antibodies against N-terminus portions of the delta receptor and against the C-terminus of the mu-receptor. Both PAG and NRM in both rats and monkey were stained by antisera against both mu and delta receptors. However, in both species, delta antisera generally labeled fibers whereas the mu antisera labeled cells. Spinally projecting NRM cells were retrogradely labeled in 3 rats and NRM sections were doubly stained for serotonin and delta receptors. When sections were examined by confocal microscopy, it was found that retrogradely labeled serotonin-stained cells were frequently apposed by varicosities positive for delta receptors.

These findings suggest immunocytochemical staining can localize opioid receptors in NRM and PAG of both rats and primates. In addition, they suggest that delta opioid receptors modulate the activity of afferents to raphe-spinal serotonergic neurons.

These studies were supported by DA 05466, DA 06299, and by the Minnesota Medical Foundation.

694.4


The MPO is involved in a variety of functions including lordosis and pain inhibition. This area has an extensive projection to the periaqueductal gray (PAG). In this study we examined the effect of electrical and chemical stimulation of the MPO on the baseline activity of PAG neurons and examined the role of glutamic acid in this pathway. We recorded from 88 PAG neurons of 22 chloride hydrate anesthetized male rats and continuously monitored the arterial blood pressure. Electrical stimulation (50-200 uA) was applied to the right and left MPO, and 85% of the cells showed a response. Both electrical (50-100 uA) and chemical (dihydrate di-homocysteic acid) stimulation led to an increase in the number of responsive cells. The most effective of the chemicals was glutamic acid. When 25 rats were used to block the excitatory response of 3010 cells in the MPO, a significant reduction of the baseline activity of PAG neurons at 4, 5, 10, 15, 20 and 30 min was observed. A mean firing rate of 4.8-11.8 Hz, inhibited cell by a mean of 8.45±1.41 Hz, and unsensitive cells had a mean firing rate of 8.75±1.33 Hz. The majority of the cells that were excited by MPO stimulation were also excited by nociceptive pinch of the tail. There was no difference between the response of cells that were inhibited by MPO stimulation and their response to noxious stimulation. In the majority of animals (18/22), MPO stimulation produced a deppressor effect that ranged between 5 and 30 min with a mean of 15 ± 2.3 min. A pressor effect was observed in 1/22 animals with an increase of 19 min. It is concluded that MPO stimulation has a significant effect on the activities of PAG neurons, which is possibly glutamic acid mediated, and produces a decrease in the blood pressure. Supported by NIH grants NS20643.

694.6

CAUDATE NUCLEUS ACTIVATION IN RESPONSE TO INTENSE PAINFUL STIMULATION IS OBSERVED USING FUNCTIONAL MAGNETIC RESONANCE IMAGING. A. DiBenedetto, J. B. Rebec*, J. H. O'Donovan, L. Antignus, C. A. Ernst, J. K. Counsell**. Depts. of Psychology, Radiology, Neurosurgery, and Neurobiology, Physiology and Behavior; Univ. of Calif. Davis, Davis, CA 95616.

The basal ganglia are believed to play a role in nociception and pain. Electro-physiological studies in cats have suggested that activation of the caudate nucleus is due to response to high-intensity electrical stimulation of the inferior dental nerve sufficient to elicit the jaw reflex opening (Lidicky et al., 1979, Brain Research Bulletin 4, 9-14).

In this study we examined caudate activation in humans during intense painful stimulation. In 5 male volunteers 2 levels of painful electrical stimulation were assessed, as well as a non-painful brush stimulus. During the 4.5 minute scanning sessions, 32 second periods of stimulation were alternated with 32 second periods of rest. The first stimulus level, which was painful but tolerable for the entire scanning session, was selected by the subject. The mean intensity of this 2 Hz stimulus was 14.8 ± 0.0 mA. The second level was a 100 Hz 50 mA stimulus. This stimulus was administered after subjects were anesthetized with 0.3% isoflurane and paralyzed with Vecuronium 0.15 mg/kg. Anesthetized subjects were also scanned during lower level electrical and brush stimulation. Functional images were obtained using a 1.5 T GE scanner with a birdcage RF coil designed for whole-volume brain imaging. A gradient echo pulse sequence was used with a TE of 40ms and a TR of 2 sec. All time course data was correlated with a boxcar function and a correlation threshold of 0.35.

Caudate activation was not seen in anesthetized or unanesthetized scans of lower level electrical or brush stimulation. However, during the intense electrical stimulation of anesthetized subjects bilateral caudate activation was seen in 4 of the 5 subjects. Consistent activation of primary and secondary somatosensory cortices was also observed.
PAIN: PATHWAYS AND MODULATION

694.7 EFFECTS OF ANTICIPATION OF VISCERAL PAIN ON THALAMIC ACTIVITY IN IRITABLE BOWEL SYMPTOMS AND NORMAL SUBJECTS. D.R. Silverman, H. Imamura, J. Markham*, M.E. Phillips, W. Blalock, E.A. Mayer. Deps. of Medicine and Nuclear Medicine, UCLA Medical Center and West LA VA Medical Center, Los Angeles, CA, 90024.

Anticipatory anxiety is prominent in many patients with irritable bowel syndrome (IBS) and is hypothesized to facilitate perception of visceral pain. The current study was designed to examine the relationship of anticipatory visceral pain and visceral pain perception in IBS patients. Subjects were 19 IBS patients and 19 age- and gender-matched healthy controls. Anticipatory visceral pain was assessed using a simulated rectal balloon procedure. The pressure threshold to reach pain was found to be significantly higher in the IBS group. In addition, IBS patients demonstrated a greater decrease in heart rate following the ingestion of a rectal balloon solution compared to controls. These findings suggest that anticipatory visceral pain may be a contributing factor to the high prevalence of irritable bowel syndrome in the general population.


Skin irritation triggers an enhanced pain sensitivity in surrounding un-injured skin (secondary hyperalgesia) due to heterotopic central sensitization. Repetitive stimulation of nociceptors at frequencies > 0.5 Hz has been shown to result in the phenomenon of "wind-up". This is thought to be a key mechanism in the development of hyperalgesia. The aim of this study was to investigate the possible changes of perceptual wind-up in the area of secondary hyperalgesia. Secondary hyperalgesia was produced by punctate mechanical stimuli or the injection of capsaicin (40 µg). Sensitivity to mechanical stimuli using cotton balls, calibrated von Frey hairs, and pin was tested at 15 mm distance from the injection site. Blunt von Frey hairs (0.5-100 g) were used to test for perceptual wind-up.

Capsaicin evoked strong burning pain and a laterally shift of S1 function (> factor 3) in the area of secondary hyperalgesia; capsaicin was also more painful than non-noxious mechanical stimuli even though both were painful. Though the pain ratings increased in the area of secondary hyperalgesia, the extent of wind-up remained unchanged. Significant wind-up was only observed in the pin prick receptive field at the 0.5 s frequency (see Fig.).

We conclude, that the adjacent injection of capsaicin causes a pronounced enhancement of cutaneous pain sensitivity to punctate mechanical stimuli, but no equally pronounced change in wind-up to repetitive stimulation.


Present electrophysiological studies allow for rather sophisticated information concerning the relationship between the stimuli parameters used for routine clinical nerve conduction studies (NCs), with little consideration given to the effect of these parameters on patient discomfort. Previous studies have suggested that some procedures may excite NCS as aversive or painful. This study was designed to examine the effect of stimulus duration and stimulus intensity on the perception of pain in individuals who have never undergone standard NCs. Ten healthy volunteers underwent an anterior cutaneous sensory conduction technique to the index finger of one hand in twenty asymptomatic subjects. For different stimulus durations (0, 100, 200, 300, 500, 1000 µsec) were compared. Stimulus current (mA) was adjusted for each subject to 20% supramaximal at each stimulus duration. Immediately following each stimulus, subjects rated the severity of pain perceived using a visual analog scale (VAS) ranging from no pain to the most intense pain possible. In addition, the State-Trait Anxiety Inventory was administered to each subject before and after testing. None of the stimuli were rated as more than mildly painful. Mean pain ratings for all stimulus durations were in the lower range of VAS perception and pain rating were found to be inversely related. Shorter stimulus durations resulted in significantly higher pain ratings. There was no significant correlation between pre- or post-test anxiety scores and pain ratings. Results of this study may provide information which is useful in decreasing pain perception and anxiety associated with clinical NCs.

694.11 INHIBITION OF PLASMA PROTEIN EXTRAVERSION BY 5-HT, AGONISTS IN RODENT DURA MATER: PHARMACOLOGICAL CHARACTERIZATION USING PRIMARY ANTIGEN KNOCKOUT MICE. C. Weaber, X.-J. Yu, N. Caruana, K. Scrace, R. Hen, J.E. Macor and M.A. Mokrant. Massachusetts General Hospital, Charlestown, MA 02129

5-HT(A2) agonists have been shown to inhibit plasma protein extravasation in the dura mater of rodents (a model of migraine). However, the potency of some of these drugs in this paradigm does not correlate with their affinity at 5-HT(A2) receptors. We investigated the 5-HT(A2) receptor subtype mediating the effects of these agents, we have used 1) a specific 5-HT(A2) agonist in guinea-pigs and 2) knockout mice lacking 5-HT(A2) receptors. Peripheral-astrocytoidatized animals were injected with [3H]BPA (300 nM) and killed 15 min after injection. The size of the retinal ganglion is significantly larger than in the untreated mice. Following intracardiac saline perfusion, the left and right retina were harvested and the ratio of extravasated [3H]BPA (stimulated/unstimulated) measured. This ratio was close to 1.7 in vehicle-treated mice. Complete inhibition of extravasation (ratio close to 1.0) was observed in both guinea-pigs and mice when sumatriptan (0.75 mg/kg), CP-122,288 (1 mg/kg) and 5-CT (1 mg/kg) were injected i.v. 10 min prior to trigeminal stimulation, the selective 5-HT(1A) agonist CP-91,129 (14 µmol/kg) was effective only in mice. In guinea-pigs, pretreatment with GR-127,935 (0.1 mg/kg, i.v., 20 min prior to agonist) reversed the effects of sumatriptan, but did not affect those of CP-122,288 and 5-CT. In guinea-pigs, sumatriptan and CP-122,288 were inactive, while CP-122,288 and 5-CT were potent as in wild-type mice to inhibit extravasation. These results suggest that CP-122,288 may serve as a selective 5-HT(1A) receptor agonist for inhibition of dural protein extravasation in rodents. In contrast CP-122,288 and 5-CT may act via a presently unknown receptor; the hypothesis in agreement with the fact that their potency in this model is much higher than expected from their affinity at 5-HT(A2) receptors.


Antagonists of the NMDA receptor are potentially useful neurotherapeutic agents for prevention of opiate tolerance, alleviation of neuropathic pain and prevention of neurodegeneration in stroke and brain trauma. However, the ability of these agents to reversibly injure or kill neurons in rodent brain and to produce psychic "emergence reactions" in humans has raised concerns about their clinical use in humans. Over the past several years we have been studying the neuroprotective effects of these adverse CNS side-effects and have proposed that they might represent different manifestations of the same toxic process. Here we report that the neurotoxic effects of NMDA receptor-mediated analgesia can be significantly reduced by pretreatment with specific NMDA antagonist, MK-801, is prevented by several 5-HT(1A) and 5-HT(2A) receptor agonists in a dose dependent fashion. The dose of MK-801 used (0.5 mg/kg) causes a reversible neurotoxic reaction "wind-up". In contrast, in specific cerebrocortical neurons in 100% of treated rats. The E90 (dose that reduced the mean number of vacuolated neurons by 50%) for the 5-HT(2A) agonists tested were clonidine (0.044 mg/kg), guanabenz (0.21 mg/kg), lorexidine (0.38 mg/kg), pindololodine (1.3 mg/kg) and xylazine (2.4 mg/kg). In a separate experiment, the psychotomimetic effects of NMDA antagonists, then co-administration of an 5-HT(2A) agonist with an NMDA antagonist might prevent the NMDA antagonist from inducing either of these adverse side effects. This would enable NMDA antagonists to be used more safely for alleviation of neuropathic pain (unresponsive to opiates but possibly responsive to 5-HT(2A) as well as NMDA antagonists) and to enable opiate analgesics to be used without addiction liability in the management of other states of chronic pain. Supported by DA07261, DA 05072, AG 11555, NARSAD Established Investigator Award, RSA MH 38894 (JW) & Scottish Rite Benefvolent Foundation's Schizophrenia Research Program (NBF).
965.1 CORTICAL DYNAMICS OF WORD RECOGNITION IN NORMAL AND DYSLEXIC SUBJECTS: A NEUROMAGNETIC STUDY. R. Salmelin1,2, F. K美女, and K. Uutela1. Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, and Dept. of Psychology, Univ. of Helsinki, 00170 Helsinki, Finland.

We explored spatiotemporal cortical organization during word recognition in 7 normal subjects and 5 subjects with a documented history of developmental dyslexia, employing a whole-head neuron magnetic resonance imaging (Neuromag-122). All subjects were behaviorally trained to read, orally read for naming, and to digit span forwards and backwards.

Subjects viewed 2-7 letter words for 500 ms (centered, 4 deg visual angle), once every 3 s. Four categories alternated randomly: concrete and abstract Finnish words, pronounceable pseudowords without meaning, and unpronounceable, meaningless letter strings, against the same amount of consonants and vowels as in the other classes. The subjects were instructed to say aloud the word (kairavin [giraffe]) whenever it appeared. Activation of primary visual areas was accompanied by lateral occipitoparietal signals within 200 ms after stimulus onset, showing a pronounced right-hemisphere dominance in all dyslexics, in contrast to the left-hemisphere dominance observed in 5 of 7 normal subjects. The occipital areas remained active up to 800–1000 ms. Concrete words elicited strong left frontaltemporal activation after 500 ms in normals, but much weaker signals in dyslexics. Dyslexia may thus be associated with abnormal right-hemisphere dominance of early cortical analysis and reduced post-lexical activation.

965.3 CORTICAL CONTROL OF VOCAL FUNDAMENTAL FREQUENCY DURING SINGING. D.W. Perry1, R.J. Collette1, and A.C. Evans1. Montreal Neurological Institute, McGill University, Montreal, QC, Canada H3A 2B4.

During singing, one must consciously control vocal fundamental frequency in order to produce intended musical pitches. In the present experiment, 13 subjects performed 3 experimental tasks, and changes in FBF were measured using transcranial Doppler sonography and the water method. In the singing condition, they vocalized a single pitch repetitively, repeatedly changing pitch, or increasing pitch gradually as fast as they could comfortably on a single breath. In the playback condition, they listened to a recording of their singing. In the whispering condition, they listened to the same recording, while whispering "ah" at the same rate. In comparison to playback, significant increases in CBF were observed during singing in the supplementary motor area (SMA), the precentral gyrus, the insula/operculum, and the cerebellum. Increases were also observed in the right primary auditory cortex, and bilaterally in occipital cortex. These findings replicate those observed previously during singing in comparison to perception of complex tones (Perry et al., 1993, Soc. Neurosci. Abstr. 19, 843). In comparison to whispering, increases in CBF were observed only in the inner face of the precerebellar operculum, the right primary auditory cortex, and the cerebellum. In comparison to playback, increases during whispering were observed only in the inner face of the precerebellar operculum. These results suggest that motor cortex in the inner face of the precerebellar operculum may be particularly crucial to the cortical control of vocal fundamental frequency, i.e. for adjustments of vocal fold length by the intrinsic laryngeal musculature. They also provide further confirmation for the hypothesis that right auditory cortex may be preferentially involved in monitoring the pitch of one's own voice during singing.

965.5 COMPARISON OF CORTICAL AREAS ACTIVATED BY PRIMARY AND SECOND LANGUAGES IN HUMAN BRAIN USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI). K. Ko1,2,2 J. Heschl2, R. DeFon2, N. Reubi1, and K. L. M. Leib1. Dept. of Neurology and Radiology, MSKCC; Dept. of Psychology and Neuroscience, Cornell University Medical College, New York, NY 10021.

Fluent second languages can be acquired throughout life, providing language learning tasks that are taken place during critical periods. The neural mechanisms involved in the development of multilingual capabilities after childhood are not understood. In order to understand the brain mechanisms involved in the development of multilingual capabilities after childhood, we used fMRI techniques to study the cortical regions activated during performance of language tasks, in particular tasks showing bidirectional activation in the left hemisphere during early adulthood. Functional brain images were obtained using a GE Sigma 1.5 Tesla scanner. Using a planar coil for the primary language capability (American University, NIMH) using a general echo planar sequence with an in-plane resolution of 1.56 x 1.56 mm. Slices 4.7 mm axial slices parallel to the AC-PC line were spaced 1.6 mm apart to image visually the entire central cortex. Twice over a period of 10 minutes, each subject underwent fMRI scanning as they read text, read verbally-encoded numbers and performed calculations using verbally-encoded numbers in Korean and English. A voxel-by-voxel, multivariate statistical analysis with a replication requirement identified the locations of active sites associated with all tasks. Cortical regions thought to have similar activations irrespective of the task were identified. However, within the regions generally considered language-related (presumably Broca's and Wernicke's areas), adjacent areas of activation were reproduced in the right hemisphere, which appeared to be unique to Korean and English respectively. These results suggest that the mapping of primary and secondary language areas may be variable at the level of the central cortex when the second language is acquired after childhood. This could reflect recruitment of neighboring cortical zones or subdivision of language-specific cortex acquiring a second language. (Supported by William T. Morris Foundation Fellowship (KHK), MSKCC (JH, RD), and CV Starr Foundation (NR)).

965.6 HIGH-FREQUENCY OSCILLATIONS INDICATE CELL-ASSEMBLY BINDING IN HUMANS. N. Birbaumer1, W. Lutzenberger & F. Pulvermüller. Institute of Medical Psychology and Behavioral Neurobiology, Univ. Tuebingen, D-72074 Tuebingen, Germany.

EEG and MEG-synchronized responses in the range of 20 to 45 Hz indicate “binding” of cell-assemblies because they reliably appear at the human neocortex if meaningful information is processed. Three experiments with healthy volunteers (N=15 in each experiment) demonstrate that (a) meaningful words and meaningless pseudowords carefully matched for word length and frequency, (b) words with motor and sensory content and, (c) regular Gestalt- and irregular visual patterns presented in different visual fields. Changes in EEG power of electrode arrays were analyzed using current source densities based on spherical spline interpolation in order to eliminate artificial influence from reference electrodes. Meaningful words elicited 30Hz gamma responses in the left perisylvian cortex only, meaningless showed no synchronized responding in any frequency range (3.5-100Hz). Words with motor content elicited 30Hz responses in left frontal regions, words with visual content 35Hz responses in both parieto-occipital cortices. Changes from chaotic to Gestalt-like visual patterns elicited 40Hz oscillations in the respective cortical representation of the visual field of the occipital lobe. The results cannot be explained by effects of harmonics of changes in alpha activity.

Supported by the German Research Society (DFG).

965.7 THE LEFT FRONTAL OPERCULUM IN PHONOLINGUISTIC PROCESSING: CONVERGING EVIDENCE FROM PET AND THE LESION METHOD. J. Fiez, A. Damasio, J.T. Raiffa, S. Raifer, and R.W. Davidson. Department of Psychology, University of Iowa, Iowa City, IA.

PET studies in normal subjects have provided evidence that a region at the junction of the inferior frontal gyri and the anterior insula is important for specific types of phonological processing (1-2, 3-4, 5). Here, we report converging evidence on this finding using the lesion method. Eleven target subjects with circumscribed left frontal opercular lesions were identified in our patient registry. The target subjects and 11 control matched controls for age, sex, and education were studied using tasks derived from the PET studies in normals. We hypothesized that patients would be impaired on tasks associated with functional activation in the left frontal opercular region, but would perform normally on tasks associated with activation of the right hemisphere. Relative to the controls, the target patients were: 1) impaired at reading nonwords (p<.004) and low-frequency words with exceptional orthographies (e.g., 'chaos') (p<.008); but read other words within normal limits (p>.05); 2) impaired on phonological processing tasks (does a word contain a long vowel sound, e.g., 'the a's in LAME') and both visual (p<.002) and auditory (p<.02) presentation; but were normal on non-target task (p>.05) on an orthographic detection task (does a word contain an "ascending" letter, e.g., "a") with both visual and auditory presentation; 3) impaired at remembering 5 words for 60 s (p<.05). The target subjects were normal on a rhyming task (p>.05). These findings provide strong additional evidence that regions within the left frontal operculum are part of a system that makes specific and critical contributions to phonological processing.

695.7

ACTIVATION OF LEFT SUPERIOR TEMPORAL SULCUS WITH FUNCTIONAL MRI OF SENTENCE PROCESSING. S. L. Small\(^1\), D. C. Noell\(^1\), C. A. Perfetti\(^2\), and W. Schneider\(^3\) Departments of \(^1\)Neurology, \(^2\)Radiology, and \(^3\)Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

We used functional MRI to investigate the unique neuronal processing characteristics of sentences. Five right-handed subjects participated. Word sequence was presented at the end of each sentence ending with a period. Subjects pressed a button at the end of each grammatical meaningful sentence. In the control task, sequences of false font strings were presented one string at a time, with each button press ending with a period. Button presses were performed with the left hand and were equalized across conditions.

Within an oscillatory stimulus presentation paradigm, active and control tasks were alternated every 4 min. Sixty 3-T sagittal spiral k-space images were obtained in each hemisphere with a 1.5 Tesla scanner using a pair of parallel 5-inch temporal surface coils. The head was restrained with a bite bar or a clamp. A gradient echo pulse sequence used 4 interleaved slices to provide 2.7 x 2.7 mm resolution over an 18 cm FOV. Each slice was acquired 8 times during each 30 sec interval (total 64).

The activation threshold for each voxel was set to exceed correlated r = 0.5 with a reference (time) waveform corresponding to the temporal pattern of stimulus presentation. The main regions of activation were the left inferior frontal gyrus and superior temporal sulcus (STS). Most subjects had a smaller degree of activation in homologous regions of the right hemisphere. Some subjects also had activation in the left angular gyrus or premotor cortex. The focus of activation in the left STS, about two thirds of the distance from the temporo-occipital tip to the temporo-occipital junction, is not identical to the region of the superior temporal gyrus implicated in most lesion studies as important for comprehension.

(Supported by NIH DC00565)

695.8


The effects of increasing difficulty of a cognitive task on functional brain activation have not been examined. There has been argued that prefrontal activation in certain cognitive tasks (e.g. working memory tasks) may be due to mental effort alone. If this hypothesis is true, then a cognitive task that does not recruit prefrontal cortex might be expected to do so when made more difficult. We studied 10 normal subjects using event-related functional MRI during a spatial location/motor rotation task in which the subjects were required to match a target figure to one of two spatially rotated figures. Difficulty was varied for each subject either by increasing the number of mental rotations required to solve the task (n=6) or by increasing the presentation rate of the stimuli (n=4) until average subject accuracy decreased by approximately 80% to 70%. Scanning was performed at 1.5 T over 16 contiguous 5mm axial slices (TR=2000 msecs, TE=60 msecs). Regions of activation were identified by correlation with task above a threshold of p<.001, and signal change was assessed within regions of interest. Task-correlated signal change was found in bilateral superior parietal lobule (Brodmann's area 7) and bilateral occipital cortex (Brodmann's area 19) in all subjects. As task difficulty increased, an increase in percentage signal change and activation volume was seen in these regions. Activation in prefrontal cortex was not noted. These findings provide insight into how the brain responds to increasing task difficulty and do not support the hypothesis that mental effort alone results in the prefrontal cortical activation seen in working memory tasks.

Supported by the McDonnell-Pew Program in Cognitive Neuroscience and NIH grant S-18506-194802.

695.9

AN EXPLANATION OF CONSCIOUSNESS. Eugene M. Brooks, M.D.* 1528 Tator Ctr., Bloomfield Hills, MI 48020.

Consciousness is postulated to be a rapid sequence of multiple, discrete events which give the impression of being a single entity. It is based on "qualitative cores" which are described as the direct or intercalary aspects of nerve cells. The cores constitute the unique essences of the "five senses", the emotions, and other forms of consciousness. Nerve impulses from these cells progress "upward" and are processed. Processing includes monitoring, selecting, screening, and combining under the control of higher centers. The higher centers are driven by pleasure or displeasure in conjunction with previously formed hierarchies. Hierarchies "compete" for the attainment of consciousness and are stored in memory. Mechanisms for rapidity of action, including "synchronized" and "abstracting" are proposed.

Nerve impulses do not create consciousness. They are consciousness experienced from the "inside." The physical or chemical elements which produce consciousness may already be known but are not recognized as such because as observers we are on the "outside." It becomes clear that theories of consciousness must be based on physical or chemical elements but until that level of explanation is reached it is fitting to rely on an anthropomorphic (a homunculus). The present paper applies not only to consciousness but also to perception, meaning, attention, curiosity, and a form of learning.

CIRCUITRY AND PATTERN GENERATION

696.1

CENTRAL GENERATION OF DIRECTED MOVEMENTS IN LOCUSTS. A. Hermann* and G. Laurent. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The degree to which insect limb motor control requires movement-related sensory feedback has been debated for decades, on the basis of experiments on locusts. We developed an insect preparation to study the neural control of scratching. We recorded fictive motor patterns for scratching by the middle leg and hindleg of locusts.

Locusts were immobilized between the prothoracic and mesothoracic ganglia used the middle leg or hindleg to scratch each of several sites that were stimulated mechanically. Thus, the head ganglia are not required for scratching. The hindleg could scratch 1) the ear, 2) the ventral or posterior hindleg coxa, and 3) the posterior abdomen. The middle leg could scratch the anterior hindleg coxa. The 3 types of hindleg scratching were readily distinguished in plots of tibial extension versus femoral elevation. Electromyographic recordings demonstrated that during scratching, in contrast to walking, tibial extensor muscle activity occurred partly during trochanteral levator muscle activity.

In several animals, we cut all nerve branches that provide motor innervation to the middle legs and hindlegs, while leaving intact the sensory innervation of the ear, abdomen, and most of the hindleg coxa. These animals could produce coordinated bursts of activity in tibial extensor and trochanteral levator and depressor motoneurons in response to mechanical stimulation of the same sites that evoked scratching in animals that could move their legs. Thus, basic motor patterns for locust scratching were generated in the absence of leg movement. These central motor patterns also displayed clear intersegmental coordination between the mesothoracic and metathoracic ganglia.

Supported by an NIH NRSA so A.B. and an NSF PFP to G.L.

696.2


Fictive rostral scratching is produced in a spinal, immobilized turtle by gently mechanical stimulation of the ipsilateral (ipsi) midbody shell bridge (J. Neurophysiol. 51: 1517, 1985). Ipsilateral scratching output normally includes bursts of hip flexor motor activity that rhythmically alternate with bursts of hip extensor motor activity. Occurrence of high powered photic cycles, "circadian rhythms" coupled to the alternation of spontaneous activity and quiescence, suggests a spontaneous occurrence of variation of fictive rostral scratching. There is no quiescent period between the end of one hip flexor burst and the beginning of the next hip flexor burst.

Stea et al. (J. N. Sci. 156; in press, 1995) described fictive rhythmic bursting of ipsi hip flexor activity, contralateral shell bridge stimulation elicited rhythmic bursts of ipsi hip extensor activity. We added to these findings with the observation that the number of bursts of hip flexor motor activity that rhythmically alternate with bursts of hip extensor motor activity. In this hemisected hindlimb enlargement preparation, the left halves of the D7 segment and the left half of the D8 segment are removed. In this preparation, ipsilateral shell bridge stimulation produced rhythmic bursts of hip flexor activity, contralateral shell bridge stimulation elicited rhythmic bursts of ipsi hip extensor activity. We observed that these findings with the observation that the number of bursts of hip flexor muscle activity that rhythmically alternate with bursts of hip extensor muscle activity.
96.3 PURINERGIC MODULATION OF SWIMMING MOTOR PATTERN IN ZENOPUS EMBRYOS. N. Drost, and D. Gilley. School of Biological & Medical Sciences, University of St. Andrews, KY16 9NL, U.K.

Although sensory inputs evoke and modify motor activity in the Zenopus embryo, the motor pattern for swimming follows an inherent dynamic progression in their absence. The frequency of swimming gradually falls throughout an episode until activity spontaneously ceases. The mechanisms underlying these time-dependent changes remain unknown.

Applications of the P2 receptor antagonist, pyridoxal-phosphate-6-adenozine-2',4'- diphosphate (1mM), shortened swimming episodes by 84±7% (n=4), suggesting that ATP may be released during fictive swimming. Adenosine added at 10-100mM did not alter the length of swimming episodes (106±21% vs 106±21% (n=6). Use of 1,2-methylene-ADP, to block conversion of AMP to adenosine by ectonucleotidases, also lengthened swimming episodes (by 26±15%, n=5). Naturally released ATP may thus be hydrolyzed to adenosine which could then oppose the actions of ATP during fictive locomotion.

The mechanisms underlying these effects of ATP and adenosine have been investigated in preliminary studies. Adenosine appears to: 1) elevate an ion channel directly; or 2) cause presynaptic inhibition. However, whole cell patch clamp recordings showed that ATP reduced voltage-gated K+ currents by 12±6% (n=11), while adenosine reduced voltage-gated Ca2+ currents by 14±2% (n=6). With the spinal motor circuitry, release of ATP may enhance excitability during fictive swimming by reducing K+ currents whereas reduced formation of adenosine, by ectonucleotidases, may do the opposite by gradually reducing Ca2+ currents. Time-dependent modulation of voltage-gated currents by ATP and adenosine may thus underlie the dynamic regulation and self-termination of the swimming motor pattern.

96.5 THE CIRCUIT LOCUS OF THE BEHAVIORAL CHOICE BETWEEN SWIMMING AND SHORTENING IN THE LEECH. B. R. Shaw, A. C. Petronis, Jr., Dept. of Biological Sciences, University of California, San Diego, La Jolla, CA 92037-0507.

We are studying the neuronal basis of the behavioral choice between two incompatible behaviors in the leech, swimming and the whole-body shortening reflex. An advantage of studying these behaviors is that the neural circuitry is particularly well understood. It is made up of a hierarchy of identified neurons: head brain interneurons influence segmental swimming-generating interneurons, which activate the oscillator interneurons that make up the CPG for the swimming motor neurons.

At a behavioral level, the shortening reflex dominates swimming: swimming is prevented during shortening and shortening can interrupt swimming. In correspondence with this at a neuronal level, the cells 204—the motor-neurons of the swimming-generating interneurons—are strongly inhibited during shortening. The cells SE1, ‘swim excitor interneurons’ in the brain that strongly excite the cells 204, show an opposite effect: they are excited during shortening. This means that during shortening, the cells SE1 can have an inappropriate excitatory effect on the cells 204 that may partly explain behavioral inhibition to 204. This suggests that the cells 204 act, at least in part, as the 'decision-site’ where the signals that promote swimming and suppress swimming are integrated. It also suggests that the cells 204 are examples of interneurons that are dedicated to a single behavior—swimming—while the cells SE1 may be multifunctional interneurons that participate in more than one behavior.

To further test these hypotheses, we will record from other swim circuit interneurons, like the osculatory cells, to determine their role in shortening. Supported by NIGMS training grant GM0107 (BKS) and NIMH research grant MH44356 (BRR).

96.6 GENES THAT CONTROL A MOTOR PROGRAM PERIOD IN C. ELEGANS. K. Neusser, D. W. Liu, and J. H. Thomas, Dept. of Genetics, Univ. of Washington, Seattle, WA 98195.

Periodic activation of a motor program is controlled by a neuronal pattern generator. Electrophysiology of some neuronal pattern generators, such as in Tritonia and Panulirus, has been extensively studied. However, the genetic basis of such pattern generators is not understood. Recently, we have found that C. elegans defecation is controlled by a temperature-sensitive neuronal pattern generator. With the exception of its periodicity (45 seconds), various properties of this clock are similar to those of a circadian clock. The defecation motor program consists of a stereotyped series of three muscle contractions, called pBoc, alkoc, and Exp. To investigate how the defecation cycle period is genetically controlled, we screened approximately 3,000 gene disruptions of the period genome and reexamined existing mutants. We identified mutations in 12 genes that can cause abnormal defecation cycle periods. These defecation cycle period (dec) mutations fall into two major groups, short cycle (dec-) and long cycle (dec+). Detailed characterization of these mutants revealed the following points. 1) Most dec mutations affected the cycle period quite differently at 20° and 25°. Because of their high frequency of isolation, we think most of the mutations affect temperature compensation rather than causing thermolabile gene product. 2) Mutations in the fir-1, fir-2, fir-3, and fir-4 genes (Katusara. et al. Genetics 136 145) showed a very short mean cycle period. The short cycle period correlated strongly with weakened pBoc and Exp motor steps, suggesting depletion of a factor by a high frequency of motor program activation. 3) Dec- mutations in dec-9 and dec-10 were dominant. These mutations were gain-of-function based on heterozygous deficiency phenotypes. 4) Most of the dec mutations did not affect internal timing of the motor program. However, mutations in two Dec- genes, dec-2 and dec-4, lengthened the interval between pBoc and Exp steps.

We are currently building double mutants among the Dec mutations in order to construct a genetic regulatory pathway for the clock.

96.7 A MOTOR PROGRAM ASSOCIATED WITH FEEDING IN ISOLATED CEREBRAL GANGLIA OF APLYSIA. Ray Perrins* and K. R. Williams, Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

The coordination of mouth and buccal mass movements varies in different forms of feeding-related behavior in Aplysia and indeed can occur independently. Buccal motor programs (BMPs) controlling buccal mass movements can be obtained in isolated buccal ganglia so the central pattern generator (CPG) controlling this aspect appears to reside there. Mouth movements are controlled largely by motoneurons in the cerebral ganglia and we have now discovered a neuron, the cerebral motor program initiator (C-MPI), which can induce a cerebral CPG (C-MPI) in the isolated ganglion when fired at rates of 12-26 Hz. The cycle period of the program is between 49 and 50 seconds, similar to those observed for BMPs. This program is activated by alternative firing of five motoneurons C11 and C12 as well as c-euster neurons including C4 and C-MPI itself. The cerebral to buccal interneurons CBI-2 and CBI-4, which can induce BMPs fire more powerfully during the C-MPI. When the cerebral ganglia is attached the C-MPI can occasionally evoke single cycles of BMP but more commonly has little effect on buccal output. During BMPs evoked by firing in CBI-2 and CBI-4 C-MPI receives strong rhythmic excitatory input. Thus there are CPGs in both cerebral and buccal ganglia which can control individual motor programs without the need for input from the other. However, the CPGs are connected at several sites. Altering the strengths of these connections, or decoupling, the mode allows a mechanism varying the coordination of mouth and buccal mass movements in different behaviors.

96.8 USING RNA-PCR TO DETERMINE LEVELS OF SHAKER FAMILY GENE EXPRESSION IN NEURONS AND GLIAL CELLS IN THE STOMATOGASTRIC GANGLION OF THE SPINY LOBSTER. D. Jaros*, C. L. Cole, H. E. Rodriguez, and R.M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The unique electrophysiological properties of the different identified neurons in the stomatogastric ganglion (STG) are essential for the generation of the pyloric motor pattern. To better understand the roles of identified currents in motor pattern generation, we are trying to correlate specific cellular currents with specific genes from the Shaker family of potassium channels. As a step toward this end, we have used RNA-PCR to survey unidentified neurons in the STG to determine what fraction of the neurons detectably express a given shaker family gene. As a first step toward these experiments, we have identified RNA and cDNA sequences that serve as templates in RNA-PCRs. Our primers are specific for four of the members of the lobster Shaker family genes and an alpha-tubulin gene. These experiments indicate that all of the individual neurons serve as templates in RNA-PCRs. Our primers are specific for at least 65% of the neurons surveyed. We have also used this method to determine differences in gene expression. To our knowledge, we have only been able to detect shak gene expression in glial cells. As the next step toward our goal, we have developed and are currently using quantitative RNA-PCR to determine differences in the level of Shaker family gene expression in identified neurons of the pyloric network. Supported by NIH NS25915.
696.9  
DOPAMINE (DA) AND SEROTONIN (5HT): COORDINATE DIFFERENT ASPECTS OF CONSUMMATORY FEEDING BEHAVIOR IN APELUSIA.  
E.A. Kobayashi*†, D.A. Baxter* and J.H. Byrge. Dept. of Neurobiology and Anatomy, Unic. of Texas Medical School, Houston, TX 77030.  
One of the salient features of behavioral pattern generators is their ability to be modulated by extrinsic and intrinsic factors. The mechanisms by which this modulation occurs are poorly understood however. We have begun to examine this issue using feeding behavior controlled to a similar extent by the central pattern generator (CPG) underlying consummatory feeding in Apeulius can medicate, in response to different types of sensory inputs. We have begun to examine how sensory inputs to this CPG influence its activity in a reduced preparation.  
696.10  
CHARACTERIZATION OF MOTOR PROGRAMS GENERATED IN ISOLATED BUCAL GANGLIA OF APELUSIA AND MYOPODERM CULTURES.  
A.M. Banta, J.J. Cashman, E.A. Kobayashi and J.H. Byrge. Dept. of Neurobiology and Anatomy, Unic. of Texas Medical School, Houston, TX 77030  
To provide further insights into cytoarchitecture of the buccal ganglia, we monitored patterned neural activity in isolated buccal ganglia with intracellular and extracellular recordings. On the basis of similarities in the CPGs, we have developed a semi-intact preparation to provide direct behavioral evidence correlating feeding behaviors with the actions of the CPG, its precursor DOPA, and 5HT.  
696.11  
NEUROCHEMICAL AND BEHAVIORAL ASPECTS OF TRANSMITTING NEURAL MEDIATION IN THE ESOPHAGEAL NERVOUS SYSTEM OF THE LEPIDOPHLOIINAE.  
R. Martinez, A. Campbell, G. Hamill and S. Chiel. Dept. of Neurobiology and Anatomy, Texas A&M University, College Station, TX 77843.  
To study the role of neurotransmitters in the mediating of coordination of processes in the esophageal nervous system, we isolated the posterior esophageal ganglia and observed their response to several drugs, enzymes, and neurotransmitters. We are also investigating the effect of these substances on the esophageal motility, and the role of the neurotransmitter systems in the mediation of the esophageal coordinated activity.  
696.12  
PATTERNING OF CONSEQUENCES OF NERVE INJURY IN ESOPHAGEAL GANGLIA.  
We have examined the consequences of nerve injury in the esophageal ganglia in vitro. Using this model, we have found that the ganglia reorganize their neural activity following nerve crush injury. The reorganization results in the appearance of novel patterns of activity, which are different from the normal activity patterns before the injury.  
696.15  
ROLE OF CHROMAFFIN CELLS IN THE NOCICEPTIVE RESPONSE.  
It is generally accepted that the response to noxious stimuli involves the release of catecholamines by peripheral sympathetic nerves. In this study we investigated the role of the adrenal medulla in the response to noxious stimuli. We found that the release of catecholamines is not necessary for the response to noxious stimuli. The response is mediated by the release of serotonin, which is also released by the adrenal medulla.  
697.1  
PURIFICATION AND CHARACTERIZATION OF ADULT OLIGODENDROCYTE PRECURSOR CELLS.  
J. Shi, A. M. Margiuse and B. A. Barnes*. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-1084.  
Although the extent to which demyelinated white matter can repair itself is not known, the injured brain appears to have at least some ability to generate new oligodendrocytes. It has been suggested that new oligodendrocytes may arise, in at least in part, from oligodendrocyte precursor cells that persist in the adult central nervous system. Oligodendrocyte precursor cells in adult brain divide little, if at all, whereas they divide rapidly in developing brain. Similarly, it has been reported that adult brain precursor cells in culture divide more slowly than perinatal precursor cells.  
Fundamentally, such differences between the behavior of adult and perinatal oligodendrocyte precursor cells could be the result of either intrinsic or extrinsic differences (or both). In order to answer this question and to further characterize the properties of the adult precursor cells, we have directly compared the properties of highly purified adult and perinatal precursor cells. In the present study, we have separated adult oligodendrocyte precursor cells by p50 rat optic nerve were isolated by immunopanning to greater than 99% purity. The purified cells share several properties with their perinatal counterparts: nearly all differentiated into GC oligodendrocytes in serum-free medium or into GFAP+ type-2 astrocytes in serum-containing medium. Clonal studies showed that nearly all adult oligodendrocyte precursor cells divided symmetrically in serum-free medium containing PDGF, NT-3, NGF-1 and thyroid hormone. They divided, however, at least five to ten times more slowly than the perinatal cells and the purified adult precursor cells did not revert to rapidly dividing cells in PDGF and FGF. These studies demonstrated that perinatal and adult oligodendrocyte precursor cells are intrinsically different and raise the question of whether adult oligodendrocyte precursor cells have the ability to revert to rapidly dividing precursor cells.
697.3

TRANSECTION OF DISOCIATED EMBRYONIC AND POSTNATAL RAT RETINAL CELLS IN CULTURE BY PARTICLE-MEDIATED GENE TRANSFER. E. M. Levine, L. G. Streitfeld, and J. A. Reti, Department of Biological Structure, University of Washington, Seattle, WA 98195.

The difficulty in transfection of progenitor cells and neurons is a major obstacle of gene expression studies in the mammalian central nervous system. In order to circumvent this problem, we have successfully transfected recipient cultures of dissociated E18-P2 rat retinal cells. In bioassay, DNA is precipitated on micron sized gold particles that are delivered to the cells by a high-pressure burst of helium in an evacuated chamber. To determine the effectiveness of this approach, we transfected the pGEX expression vector containing either the E.coli β-galactosidase (β-gal) gene or a 6-relyc myc epitope tag (MT) under the control of the immediate early CMV promoter. Cytotoxic detection of β-gal was done with the substrate X-gal or with anti-β-gal antibodies. The MT was detected with the 9EC10 anti-MT monoclonal antibody. Transfection of high-density coverlip cultures (initial plating density 2.5 x 10^5 cells) ranged from a few hundred to over 1000 cells. Expression of the transplanted plasmids was observed 8 hours to 9 days post-transfection. Using cell-type specific antibodies to identify the retinal cell classes, transfection was observed in most retinal neurons as well as progenitor cells. In co-transfections using the pGEX-β-gal and pGEX-MT, most cells that were successfully transfected expressed both β-gal and the MT. Therefore, this technique is amenable for gene expression studies in embryonic and postnatal rat retinal cultures.

This work was supported by funds from NINDS.

697.4

DETECTION OF GENOMIC SEQUENCE VARIATION BETWEEN NEOCORTICAL CELL LINES AND GERM LINE DNA. L.J. Cortes and J.M. Chusid*, Dept. of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, CA 92036-0636.

We are investigating the hypothesis that genomic DNA rearrangements occur during the normal development of mammalian CNS neurons. The technique we have utilized is restriction fragment length analysis (RFLA), a PCR-based method that allows selective amplification of putative recombinant forms. This method was unique to a given DNA sample (Liston et. al., Science 259: 946, 1993). With the assumption that the cells of the CNS are not homogeneous with respect to genomic DNA variability, the cell line derived from the mouse embryonic stem cells was used to detect RNA producing reprogrammed LOC in the CNS. RDA was used to assess variability differences between homozygous and heterozygous genes in the strain of animal. Was derived from adult male. The cell line contains one copy of a recombinant provirus with the SV40 large-T oncogene (LgT). A HindIII fragment of 327 bp in the liver gene, not present in the normal genomic DNA, serves as a positive control for the technique. Southern blot analysis of RDA products has shown this fragment to be selectively amplified in our experiments. Several other restriction fragments of various sizes were also selectively amplified. These RDA products may result from altered restriction fragment length created by proviral integration, spontaneous or site mutations in the cell line during culture, or normal in vivo rearrangements occurring before the cell line was established. Three approaches are being used to analyze these fragments: 1)Identification of proviral-containing products by Southern blot analysis, 2) determination of sequence homologies within-between various products and known genes; and 3) generation of RDA products from independently derived cell lines to determine which products among various cell lines have common genomic loci. (Supported by the March of Dimes, the California Tobacco Disease Related Program, and the Lucile P. Markey Charitable Trust).

697.5

A PRODUCT(S) OF ACTIVATED MICROGLIA PROMOTES CHOLINERGIC DIFFERENTIATION OF CULTURATED BASAL FOUREIN CELLS. J. A. Cordone, S. M. Jonakait, M. L. Schilling, F. Luskin, R. Wei, and L. Ni, Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102 and Dept. Anatomy & Cell Biol., Emory University School of Medicine, Atlanta, GA, 30322.

In cultures of embryonic septal nuclei with adjacent basal forebrain (SNBFP), interferon-γ (IFNγ) dramatically increases the activity of choline acetyltransferase (ChAT) by increasing the number of choline neurons (Jonakait et al., Neurosci. 12:1149). Since the target of IFNγ is ameboid microglia, we have assayed the effects of conditions that necessarily activate microglia (CM) on ChAT activity in these cultures, and have found that these CM raise ChAT activity in SNBFP cultures 15-16 fold. Efficacy of CM is dependent on the density of cultured cells, reaching maximal effects at approx. 10^5 cell/ml. IFN treatment of RAW cells is not required to obtain activity.

Cultures labeled with retroviral markers (BAG) and treated with either IFNγ or CM from activated microglia or RAW cells show a 3-9-fold increase in the percentage of cholinergic cells detected in retrovirally labeled clones. Neither NGF nor BFGF mimics this action. These data suggest that all three treatments promote cholinergic differentiation of undifferentiated precursors present in the cultures.

697.6


We utilized a strain of mice, derived from a radiation mutagenesis experiment and carrying an activity attenuated allele of the X linked enzyme, G6PD, to analyze the development of the cell lineage leading to cerebellar Purkinje neurons. Due to random X inactivation during early embryonic development, X linked genes can be used to distinguish between clonally related populations of cells in X inactivation mosaics. Following histochemical staining for G6PD activity, the numeric proportions of Purkinje cells expressing either the wild type or the mutant enzyme and the spatial distribution of these cellular phenotypes and their relation to anatomically and genetically defined cerebellar compartments was analyzed. Our data suggest that cerebellar Purkinje neurons originate from a limited pool of precursors. The size of this pool is different from the one derived from chorionic mice (e.g. Vogel & Herrup, Dev.Biol. 156, 49, 93), allowing us to deduce the relative timing of Purkinje cell lineage restriction. Our data also show that Purkinje neurons of distinct lineage are extensively intermingled within the cerebellar cortex. Together, these findings imply that genetically defined cerebellar compartments (e.g. Oberdieck et al, Neuro 10, 1007, 93) are not a simple consequence of the spatial arrangement of clonally related Purkinje cells. Rather, they suggest a role for cell/cell communication in the development of these compartments and hint to a temporal window during which such cellular interactions may take place. Supported by the BMBF, the DFG, and NATO.

697.7


Taste buds consist of several cell types, based on morphological and immunocytochemical characteristics. For example, basal, dark, intermediate and light cells can be distinguished using electron microscopy (e.g. Delay et al., J. Comp. Neurol, 1986); subsets of cells that are serotonin immunoreactive or gustducine immune can be distinguished at the light microscopic level (Bottiger et al., Chem. Senses, in press). Using mosaic analysis, we previously demonstrated that multiple progenitors give rise to this complex population of neuronal precursors; that these progenitors derive from local epithelium (Stone et al., PNAS, 1995). Further investigation now has revealed that this local epithelium giving rise to taste buds may be of either ectodermal or endodermal origin. Currently, we are addressing the question of cell lineage relationships within the taste bud. As in previous studies, we are using X inactivation mosaic mice to examine clonal relationships. These mice are transgenic, female mice that contain a marker on one of their two X chromosomes. Early in development, this results in the ubiquitous production of β-galactosidase (β-gal) in embryonic cells. However, during development, as occurs in mammalian females, one of the two X chromosomes is randomly inactivated in each cell. This process is stable and clonally heritable, and results in two cell populations in the mouse: one that expresses β-gal, and one that does not. Thus the mouse is mosaic for β-gal expression, and this mosaicism can be used for cell lineage analysis. In the taste bud, this is accomplished by analyzing taste buds that contain both β-gal+ and β-gal- cells. Statistical analysis of the relationships between β-gal expression and morphological or immunocytochemical cell types indicates which of these cell types are clonally related.

697.8


Human leptomeningeal cells (HLC) were cultured at autopsy from a 64 year old woman (6 hours after death). 10^6 HLC (in 4 ul) incubated in fast blue or premaker were placed in an aspiration pocket in the C3 dorsal columns of 40, adult, athymic male rats. Rats were perfused with 4% paraformaldehyde after 3 days (1,2,3,4, and 6 weeks). Using confocal microscopy and immunofluorescence studies, were performed using antibodies to GFAP and neurofilament protein. Cells in the spinal cord that contained fast blue, were positive for GFAP and negative for neurofilament protein were considered to be grafted HLC that differentiated with astrocyte-like characteristics. Cells with these characteristics were found in the adjacent dorsal and ventral horns of the spinal cord in the area of the implantation pocket. These cells were observed for the 8 weeks of the experiment. These data indicate that there are pluripotent cells in aged humans.
697.9
Embyronic rat hind limb cells injected into the caudate-putamen mature into fast and slow muscle fibres arranged as fascicles around chondrogenic cores. C.J. Pan, Rushmore, K.A. Rogers, W.A. Hewson* and P.A. Mennill* Dept. of Anatomy, Univ. Western Ontario, London, Ontario, Canada N6A SCI. Myoblasts obtained from embryonic day 14 (ED 14) rat hind limbs have been shown in vivo to differentiate into small myotubes which exclusively express embryonic and slow myosin heavy chains (MyHC). Unlike myoblasts obtained from later time points in development, these cells undergo limited fusion and do not express EDL MyHCs. (Dev. Genet. 14, 356, 1993). Due to these differences, we have postulated that "embryonic myoblasts" represent a myoblast lineage responsible for the formation of slow muscle fibres in vivo. To test this hypothesis, cell suspensions from ED14 rat hind limbs were stereotaxically injected into the caudate-putamen of adult rats to determine their developmental potential in an ectopic site. At two weeks post-injection, it could be seen that extensive proliferation and fusion of the myoblasts had occurred. By four weeks, the grafts were histologically similar to fetal hindlimbs. In every graft, large muscle fascicles could be seen encircling a cartilaginous core. Prior to innervation, muscle fibres typically contained peripherally-located nuclei and expressed slow, fast or both slow and fast adult MyHC isoforms. Staining with alcian blue and silver nitrate revealed that the cartilaginous cores are undergoing osteogenesis, exhibiting all of the typical stages of endochondral ossification, and are enclosed by a well developed periosteum. These results suggest that (a) ED14 hindlimb myoblasts are capable of forming both slow and fast muscle fibres when maintained under permissive conditions in vivo, and that (b) innervation is not required for the expression of adult fast and slow MyHCs. Furthermore, the organization of cells within the grafts to form osteogenic cores and muscle fascicles suggests that embryonic cells sort themselves within the grafts to generate structures similar to those seen in fetal limbs. (Supported by MDAC).

697.10
EXPRESSION OF MEF2C IN FETAL MOUSE BRAIN. D. Leitch, Y. L. Li, and K. Wehr. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510. MEF2C (myocyte-specific enhancer binding factor 2C) is a member of the MADS family of transcription factors and is expressed preferentially at high levels in human and rodent cerebral cortex. We have previously studied the distribution of MEF2C immunoreactivity in human cerebral cortex from 14 weeks of gestation to adulthood. In adult human cortex, double-labeling experiments indicate that it is expressed preferentially in inhibitory interneurons. In human fetal cortex, MEF2C immunoreactivity is localized to the cortical plate. It is not found in the ventricular or intermediate zones during the stages of fetal development that we examined, but cortical neurogenesis is nearly complete by the earliest ages that we were able to examine. We have now used in situ hybridization to examine the distribution of MEF2C mRNA in the developing forebrain of the mouse during the period from E11 to E17 when most cortical neurons are generated. Both methods indicate that MEF2C is expressed in the cortical plate and that, if any, is present in the ventricular or intermediate zones. In addition, MEF2C is detectable in other areas of the brain including the olfactory bulb, the basal forebrain, the inferior colliculus, and the cerebellum, and in some cells within the thalamus and amygdala and in scattered cells elsewhere in the brain. These results indicate that MEF2C expression is tightly controlled both spatially and temporally. In addition, the results indicate that it appears to be turned on in cortical neurons after they become post-mitotic and migrate to the cortical plate. This pattern of expression suggests that MEF2C may have a role in the post-mitotic differentiation of specific subsets of neurons.

697.11
CLONING & EXPRESSION OF ZEBRAFISH LH-2/apoptosis HOLOGRESS. T. Tsutotewa, K. Iyemura, H. Okamoto*, Dept. Physiol., KEIO univ., Sch. Med., Shinjuku-ku, Tokyo 162, Japan. LIM home domain transcription factors play important roles in cell fate determination during development in vertebrates and invertebrates. Drosophila (apoaequor), a member of this family, is involved in the establishment of dorso-ventral compartment in wing imaginal disc and in identity determination of specific sets of muscles and neuroblasts. In mammals, LH-2, a vertebrate counterpart of apoaequor, was cloned in rat, and shown to be expressed in the nervous system. We identified LH-2/zap homologs in zebrafish and examined its expression pattern using in situ hybridization. Two of them were expressed in the developing eyes and diencephalon at 10hr after fertilization, but the expression in the eyes faded by 16hr. By 28hr, their expression was restricted to many neuromeurone boundaries (e.g. between cerebral cortex and putamen, retina/medulla, and brain and hindbrain) and also to every rhombic border in the hindbrain. Double staining with in situ hybridization and an antibody which stains most of the zebrafish choroid plexus, revealed that many early stages of developing zebrafish brain (POC, DVDT and commissural tracts in rhombomeres) were formed along the regions where LH-2 mRNA was expressed. Accumulating evidences have suggested that early neurons are born along the neuromeurone boundaries and neuromeurone boundaries may express local cues for axonal guidance. The expression pattern of zebrafish LH-2 suggests that LH-2 may control either the expression of such cues or the differentiation of neurons on the borders.

697.12
DEVELOPMENTAL REGULATION OF THE PDGF-a-RECEPTOR EXPRESSION IN GLIAL CELLS AND NEURONES OF THE MOUSE CENTRAL NERVOUS SYSTEM. B. Delacourte, T. Tsutotewa, K. Iyemura and A. Barou, Van Evercooren* U314 INSERM, Hopital de la Salpetriere, 75651 Paris cedex 13, France. Several studies have provided evidence for the expression of the PDGF-a-receptor (PDGF-a-R) in oligodendrocyte progenitors (O-2A). The effects mediated by this receptor are proliferation, migration and chemotaxis. High levels of PDGF-a-A chain transcripts are detected in neurones of the embryonic and adult mouse central nervous system (CNS), suggesting that they may derive from the O-2A progenitor cells and that PDGF-a-R is involved in oligodendrocyte progenitor migration or myelinisation. In this study, we analysed the expression of the PDGF-a-R during the postnatal (P) development of the mouse CNS by in situ hybridization and immunohistochemistry on brain and spinal cord tissue sections. Between P1 and P15, PDGF-a-R (transcripts and protein) are preferentially expressed by O-2A progenitors and pre-myelinating oligodendrocytes as previously reported. At P15, these cells with an O4-phenotype are in contact with PDGF-a-R positive neurones in the cerebral cortex. At P21, this expression spreads throughout the brain and spinal cord and correlates well with the peak of myelination occurring at this time. In the mature CNS, PDGF-a-R mRNA and protein are not detected in oligodendrocytes but are found in very few glial progenitors located in the cerebral cortex or the spinal cord white matter. Neural progenitors located in the subventricular zone and the ependymal/subependymal layer of the olfactory ventricle are also PDGF-a-R positive. The most striking observation in our study is that mature neurones strongly express the PDGF-a-R. In the forebrain, PDGF-a-R positive neurones are not detected in the ependymal layer of the olfactory bulb, prefrontal and entorhinal cortex. In the cerebellum, Purkinje cells and deep nuclei neurones highly express PDGF-a-R, as well as different brainstem nuclei, e.g. the facial and vestibular nuclei. In the spinal cord, neurones of the dorsal horn and motor neurones of the ventral horn are also labelled. This neuronal expression is first observed around P7 and extends to restricted central neuronal populations. The present data demonstrate a developmentally regulated sequential expression of the PDGF-a-R early in glia and late in mature central neurones, suggesting a more extensive role for this receptor than previously described.
MORPHOMETRIC ANALYSIS OF CULTURED RAT BRAINSTEM MOTONEURONS: INFLUENCES OF GLIAL CELLS AND MUSCLES. 
J.T. Trachtenberg and W.J. Thompson. Department of Zoology, The University of Texas at Austin, Austin, Texas 78712.

Morphometric analysis of cultured rat brainstem motoneurons was performed to study the effects of glial cells and muscles on the morphology of these neurons. The results showed that glial cells and muscles positively influenced the development and differentiation of motoneurons in culture. This study highlights the importance of the microenvironment in regulating the growth and function of motoneurons.

698.3
APOTOPSIS OF SCHWANN CELLS AT THE DEVELOPING RAT NEUROMUSCULAR JUNCTION IS REGULATED BY MOTOR NEURONS
L.T. Trachtenberg and W.J. Thompson. Department of Zoology, The University of Texas, Austin, Texas 78712.

Recent research from this lab has shown that Schwann cells play critical roles in the processes of reinnervation and terminal sprouting in skeletal muscles. Terminal Schwann cells overlying denervated endplates sprout elaborate processes which guide the nerve fibers to reinnervate the terminal Schwann cell. In light of reports which suggest that regeneration and terminal sprouting are dependent on the presence of Schwann cells, we have examined the effects of sciatic nerve transection on normal Schwann cells. Within 3-6 days of nerve transection in rats, 90-100% of terminal Schwann cells covering denervated endplates disappear. The remaining, post-surgical Schwann cells, which lack terminal Schwann cells, decrease in number with time after denervation. Our findings suggest that the presence of Schwann cells is crucial for the regeneration of nerve terminals in the peripheral nervous system.
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698.7 SCHWANN CELL-COATED CARBON FILMSTANCES AS STIMULATORS OF CNS AXONAL GROWTH: 2. S lampet, T. Khan, K. Klein, O. DeVries, L. Liu, Rehak, R.C. Folkman, V.A. Harding, and Dept. of Biochemistry, Medical College of Virginia, MCV Station Box 614, Richmond, VA 23298.

Regrowth of axons in the CNS occurs to a limited extent due to the nature of the environment. We have previously utilized small diameter (5μm) carbon filaments (Amosca Thomas & Beaudet, unpublished) to induce direct regrowth of transplanted axons in the CNS. We co-cultured Schwann cells (SC) with carbon filaments in the absence and presence of 14 day old rat fetal spinal cord tissue. We utilized a human cell line (N17-17) derived from a human soft tissue sarcoma originating from a patient diagnosed with neurofibromatosis. These cells showed the expected immunoactivity to antigens known to be present on SCs. In culture, these cells displayed the typical spindle shaped bipolar morphology and aligned themselves in fascicles.

Co-culture of NF-1 cells with dorsal root ganglion (DRG) neurites demonstrated that NF-1 expressing SCs preferred the neural substrate, i.e., carbon filament, more than the surface of other NF-1 expressing cells. The ability of NF-1 expressing cells to promote axonal outgrowth is similar to that observed in vivo or in vitro conditions known to be associated with SC, made them promising candidates for interacting with carbon filaments to further promote CNS axonal regrowth.

Scanning electron microscopy revealed that the NF-1 cells interacted with the surface of the carbon filaments aligning themselves with their long axis parallel to the long axis of the carbon filaments. The surface of the NF-1 cells was covered with numerous filopodia which appeared to aid in the subcellularization of the carbon filaments. The presence of SC in the co-culture induced extensive neuritic outgrowth from the explants. Experiments to define the optimal ratio of NF-1 cells to carbon filaments in culture are in progress. These experiments are designed to manipulate the NF-1 carbon filament culture conditions so that the majority of the surface of the carbon filaments will be completely covered with NF-1 cells. In turn, these SC coated filaments should act as an effective stimulator of axonal regrowth in vivo. Supported by NS15408 (HIDV) and Rehabilitation R&D Service, DVA.


Connectivity in the retina is established by either general or cell specific rules. The maintenance of correct dendritic and axonal Arbor size plays a critical role in the maintenance of convergence ratios within the retina, which are responsible for the retina's bidirectional and sensitive vision. We have in this lab described the relevant environmental factors that control the development of retinal ganglion cells: the retinal ganglion cell axon polarization, and the density of presynaptic input available to the cell. We manipulated retinal cell density in an effort to determine the effect of cell density on the production of bipolar and dendritic Arbor size.

This study used visual form deprivation to experimentally enlarge the eye in the chick. Visual form deprivation induces ocular rotation, which results in a reduction of the normal retinal area and a reduction in RGC density of 20-30%. Ocular enlargement produced bipolar cells whose somas are 11.8% larger, whose axonal Arbor is 36.8% larger, and whose Arbor process fewer branchings and spines. The number of IPL stratifications was also found to be inversely related to the total Arbor size.

Center - periphery effects were also observed. Periphera al axonal Arbor size increased 30%, an effect that was strongest in the experimental retina. The total length of the bipolar's dendritic process was also increased in the periphery, and a positive correlation was found IPL and arbor size. These results are consistent with the results found previously for the RGC population of the chick; in both cases IPL axons increase their coverage to fill the space made available by the retinal enlargement. The relationship between Arbor size and number of stratifications suggests that in eye enlargement, growth is stimulated to such an extent that a physiological limit for either growth rate or cell size is reached and retinal coverage is conserved at the expense of IPL stratification. In this way multistratifying processes is subordinated to the maintenance of processing capability. Supported by NIH RO1 NS19245.


Regulation of survival and cell-extrinsic activity of dopaminergic, diffusely branching, diffusely interconnected neurons is an interesting and special case of affinitarget matching in the nervous system. Previously, visual form deprivation and optic nerve injury was employed to study factors controlling arborization of retinal ganglion cells (RGC). Visual form deprivation induces ocular enlargement and retinal expansion. Partial optic nerve section causes degeneration and loss of RGCs. We have reported that the growth of RGC arbors is limited in low RGC density region and branch density is increased, indicating a role of cell density and shape RGC dendritic arbors. We have adopted the same experimental paradigm to study the dopaminergic amacrine cell. Teale et al (1993) have found that the dopaminergic amacrine cells expand in expanded retinas to maintain their coverage. In this study, we examine arborization of the dopaminergic amacrine cell following partial optic nerve section.

Partial optic nerve section was performed in newly hatched chicks. Tyrosine hydroxylase immunohistochemistry was conducted on retinal thick sections in four week old chicks. In RGC depleted retinas, cell density of dopaminergic neurons remains constant and has a central to peripheral difference in control retinas, that is, cell density becomes less dense towards the periphery. Some size and dendritic Arbor are not changed. In contrast, mean branch length is increased, which corresponds to a decrease in branch number in higher order branches and no change in total Arbor length.

We conclude that dopaminergic amacrine cells do not die or change their neurotransmitter complement following early RGC degeneration. Also, a change in RGC contribution to the IPL to fewer, denser RGC arbors causes a reduction of the dopaminergic cell branching while coverage of retinal area is maintained.

(Supported by ROI-NS10254 & T32-MH19309)


We have previously shown that some of the cell bodies of septophippocampal neurons survive for weeks to months following transection of their axons. Additionally, we have also shown that some of these neurons survive for weeks to months without signs of retraction. The purpose of the present study was to examine the ultrastructure of these axons in more detail. Bilateral aspirative lesions of the femtra-formawere made between the medial septum (MS) and septal pole of the hippocampus in adult female Sprague-Dawley rats. One week later, the specimens were immersed in 2% PFA ( Freel) and placed into the fixative. Following an additional week, the animals were killed and brain sections were cut on a vibratome. Sections through the septum and the region of the lesion were immunocytochemically stained for PHA-L using colloidal gold. The hippocampal sections were histochimically stained for ACHE as an indicator of the extent of septal cholinergic degeneration. PHA-L stained sections were immunocytochemically stained for PHA-L using colloidal gold. Photographs of the light microscopic level. Regions containing labeled axons were then selected, trimmed, thin sectioned, and mounted on mesh grids. Sections were examined in a JOEL 1200EX electron microscope. The labeled axons showed a granular appearance characteristic of DAB and were unlabeled. Many of the surrounding unlabeled axons were myelinated. Due to the granular DAB labeling, it was difficult to define these axons as DAB positive. However, the number of unlabeled axons present, was in good agreement with the number of labeled axons present at least two weeks after transection and that their ultrastructure is unremarkable.


FACT1 is a novel developmentally regulated protein present in neuronal cytoplasm during brain development and is degraded to both neuronal cytoplasm and a subset of neuronal plaques in Alzheimer's disease. To further analyze the role of this protein during neuronal injury, we utilized the rat hippocampus (HL) by the entorhinal cortex (ERC) lesion and assessed FACT1 distribution with two monoclonal antibodies directed against different domains of the protein. Ablation was from the ML in unlesioned animals. By 2 days post ERC lesion there was intense FACT1 immunoreactivity spanning the denervated outer ML; the inner ML remained unaltered. We examined additional animals at 6, 15 and 30 days post lesion, during which time commissural and associative (CA) afferents from inner ML are known to sprout pathway into the denervated zone. FACT1 staining continued to be localized to the outer ML at each time point, but appeared to recede from the interface of inner ML trans-laminar expansion. We previously demonstrated rapid re-expression of the embryonic form of neural cell adhesion molecule (nCAM) in the denervated outer two thirds of the molecular layer following ERC lesion. FACT1 expression is, following ERC lesion, p300 and p340, respectively.

We believe that the developmentally specific protein in CNS injury response to injury.

In the normal adult rat brain, the posterior septal nuclear project ipsilaterally through the stria medullaris (nucleus medialis) to provide a dense innervation of calretinin-immunoreactive axons to the cholinergic neurons of the medial habenula (MHB). In this study, we investigated the plasticity of axon projection, one and four weeks after unilateral MHB deafferentation. At one week, there is a complete loss of calretinin-positive fibers throughout the entire rostro-caudal extent of the MHB. At four weeks, calretinin-positive MHB remains unchanged from controls. At four weeks, however, calretinin-positive axons and terminals of clusters are again evident in the ipsilateral MHB, yet confined to the territory of the nucleus. Immunostaining for neuronfilament-200 and GAP-43 also reveals a higher density of reactive fibers in the caudal, as compared to rostral, aspect of the ipsilateral MHB. Following injections of biotinylated dextran amine into the ipsilateral MHB at 4 weeks after unilateral deafferentation, labelled axons are evident in the habenular commissure and contralateral sm, as well as labelled somata in the contralateral posterior septum. In those animals with bilateral lesions of the sm, there are no calretinin-positive fibers found within either MHB after 4 weeks. These data reveal a remarkable degree of plasticity among calretinin-positive septal axons in the adult rat following unilateral deafferentation. We propose that the new calretinin-positive fibers found in the deafferented MHB arise from neurons in the contralateral septum which project through the intact sm and into the habenular commissure. (Supported by Queen’s Univ.)


Synaptic reorganization in rat hippocampal formation following dorsal septohippocampal pathway lesion were determined by employing immunohistochemical method with the antibody against the immature protein(GAP-43). Within the 1st week, there was a significant decrease in the GAP-43 immunoreactivity(IR) in the supragranular layer of dentate gyrus and stratum lacunosum moleculare of the CA-1 field. Between 2 and 3 weeks after lesioning, levels of the GAP-43 IR were found to increase markedly in the inner molecular layer of the dentate gyrus, coinciding with the time at which commissural-associational fibers sprout axon collaterals into dendritic portions denervated by the lesion. In the stratum lacunosum moleculare of the CA-1 field, GAP-43 IR was revealed at 1 and 2 weeks later, presumably due to the sprouting of other inputs. GAP-43 levels in the ipsilateral supragranular layer after the lesion of septohippocampal pathway did not return for 12 weeks, suggesting that ipsilateral supragranular layer did not receive terminal fibers via any other routes after dorsal septohippocampal pathway lesion. In contrast to the ipsilateral side, level of GAP-43 IR in the supragranular layer contralateral to the lesion increased during times of terminal axon sprouting, interestingly.

AXONAL SPROUTING IN THE HIPPOCAMPAL DENTATE GYRUS IN NCAM DEFICIENT MICE. LM Fujisaki and S.W. Scheff. Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536.

The hippocampal dentate gyrus has been used extensively to study plasticity in the CNS. Following partial denervation of the granule-cell layer, a morphological change occurs among the residualafferents innervating the dentate molecular layer. One aspect of this change is the growth of the commissural (CA) fibers following removal of the ipsilateral entorhinal cortex (EC). Neural cell adhesion molecules (NCAM) play a central role in developing neural systems in which they influence axonal growth, guidance, synaptic formation, neural cell migration, and peripheral nervous system regeneration. Little is known of their role in axonal sprouting in the adult CNS. The NCAM deficient mice generated by gene targeting were compared to normal mice for their ability to support reactive outgrowth of the CA fibers. Mutant and normal mice were subjected to unilateral removal of the EC and 3 days later, unilateral lesions were made. In the hippocampus, the CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres. The CA fibers were assessed at early and late times to assess changes in the sprouting fibres.

Both normal and NCAM deficient mice displayed intensified AChE staining in the dentate molecular layer following CA fiber sprouting. Analysis of the outgrowth of the CA fibers revealed robust axonal growth and no significant difference between the two groups was observed. However, NCAM deficient mice did not show any important role in brain plasticity when present, it is not necessary for all CNS self-repair. Supported by AG05144


Target survival cells were shown to increase some biochemical aspects of the development of dopaminergic (DA) mesencephalic neurons in culture. In the present study, we attempted to define the specific aspect of dopaminergic cell development that is typically influenced by survival neurons. Embryonic DA neurons of rat mesencephalon were cocultured in presence of neurons derived from either target survival or non survival cerebellar tissue. After 4 days of disobined culture in a chemically defined, serum-free medium, two aspects of the development of DA neurons. Neurons were treated: 1) morphological, the morphometric analysis of DA neurons outgrowth after tyrosine hydroxylase (TH) immunostaining (2) biochemical, the quantitative analysis of intracellular TH contents after Western blot analysis and after densimetry of TH immunolabelling in individual cells. The total length and particularly the complexity of TH+ neurons (number of branching points) were dramatically increased when DA neurons were cocultured in presence of survival cells but not cerebellar ones. To define what type of neurons was concerned, TH immunofluorescence was coupled with that of specific markers of either dendritic (MAP2) or axonal (phosphorylated neurofilament) compartment. It was thus shown that the presence of survival neurons induced highly branched axons, without changes of the dendritic arborization. Due to the culture conditions (serum-free medium), the target survival neurons themselves and not stria tals glia were accountable for this peculiar effect on the axonal outgrowth of dopaminergic neurons. Conversely, the increase in intracellular TH contents in DA neurons observed in parallel, appeared to be influenced in the same extent by the presence of either target or non target neurons.

VASCULAR PRESSURE LEVELS AND CYTOCHROME OXIDASE ACTIVITY IN THE HYPOTHALAMUS, HIPPOCAMPUS, AND CORTEX DURING ANOXIA. SYNAPTIC SPROUTING: EFFECTS OF HYPNOTREMIA. C.W. Mclntosh, J.P. Herman and C.M. Padon. Dept. of Biology and WAMI Medical Program, Montana State Univ., Bozeman, MT 59717 and Dept. of Anatomy and Neurobiology, Univ. of Kentucky Health Science Center, Lexington KY 40536.

We have previously shown that unjured magnocellular neurons on one side of the rat hypothalamus increase significantly in the medial hypothalamus (N.L.) following, destruction of the contralateral NL to the NL. These aspects involved in the mechanism of control of the blood pressure response, have been elucidated. We have shown that neuroinflammatory factors released by the inflammatory response following, brain injury or ischemia, may also play a role in the increase in blood pressure. In this study, we investigated the effects of severe hypotremia, induced by administration of Hypoxia in the rat. A single intraperitoneal injection of Hypoxia was used to induce hypotremia. The animals were then sacrificed at various times following, the administration of Hypoxia and the brains were removed for histological examination. The results showed that severe hypotremia induced by administration of Hypoxia was followed by a significant increase in the number of immunoreactive neurons for cytochrome oxidase in the NL. This effect was also observed in the contralateral NL. These results suggest that the increase in cytochrome oxidase activity in the NL following hypotremia may be a result of the increase in blood pressure response following, the administration of Hypoxia.
698.19


Temporal lobe epilepsy is associated with neuronal death, gliosis and sprouting of mossy fibers (ie. the axons of dentate granule cells) in the hippocampus of human and rats. This has been well analyzed in the model induced by kainate injection (see Repirot et al., '95 J. Neurobiol. 26, 413 for a review).

In the present study we show that immunoreactivity and mRNA hybridization of tenascin-C (an extracellular matrix glycoprotein with repulsive properties) increase in the hippocampus of rats treated with kainate. The increase of tenascin-C was particularly striking in Ammon’s horn, where tenascin-C probes and antibodies labelled reactive astrocytes. Tenascin mRNA expression was also induced in granule cells and protein immunoreactivity appeared in plasma membranes of their axons, the mossy fibers. Tenascin-C immunoreactivity remained unchanged in the molecular layer of epileptic rats.

It is interesting that increased tenascin-C immunoreactivity was observed within zones in which axonal regeneration does not occur (the CA3 area in kainate treated animals) whereas zones in which reactive synaptogenesis occurs (the molecular layer) were devoid of tenascin-C immunoreactivity. We may infer from these results that tenascin-C impedes the terminal sprouting of mossy fibers in CA3 of kainate-treated rats.

698.20


Ablation of mossy fibers (MF), which are the axons of dentate granule cells, occurs in epilepsy and can be experimentally induced by intraventricular injections of kainic acid (KA). It has been reported that MF sprouting occurs more rapidly after bilateral KA than after unilateral KA. A possible explanation for this difference is that commissural afferents from the contralateral dentate gyrus sprout and compete with the ipsilateral mossy fibers, thereby retarding their sprouting.

In the present study we investigated the role of the commissural afferents from mossy fiber sprouting to the hilus of the hippocampus in KA-induced sprouting. Animals received either bilateral KA or unilateral KA and were killed 2, 3, or 4 weeks after surgery.

The hippocampus was separated into three groups: hilar KA, bilateral KA, and unilateral KA with KA placed unilaterally. We tested the degree of hilus mossy fiber sprouting in the KA vs. the controls and found that the KA was significantly reduced in the KA group compared to the control group. This suggests that the degree of hilus mossy fiber sprouting is dependent on the number of mossy fibers that reach the hilus.
699.5

ANTIDOPYTIC ANTIBODIES TO GM1 ENHANCE GROWTH FACTOR STIMULATED NEURITE ELONGATION. M.J. Bignall* and W.L. Matthews, Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

We have tested the ability of antidopytic antibodies to GM1 (AIG Ma) to stimulate neurite outgrowth in a biosensor using cultures of human neuroblastoma cell lines. AIG Ma antibodies were used to stimulate neurite outgrowth using a modified version of the microtubule assay. Neurite outgrowth was measured for each neuron: the length of the longest neurite, the number of primary neurites and the number of branch points. The AIG Ma antibodies were cultured in the presence of AIG4, AIG5, AIG20, control antibodies or GST conditioned media. To test the dependence on growth factors, these experiments were conducted in one of the following media preparations: a) no added growth factors; b) a suboptimal concentration of NGF; c) a suboptimal concentration of NGF plus N2 (insulin, transferrin, progesterone, putrescine, selenium).

The AIG Ma have the ability to promote neurite elongation under all conditions. However, elongation is most pronounced when DRG neurons are cultured in the presence of growth factors and either antidopytic antibody AIG4 or AIG20. The growth factors have a negligible effect on neurite length in the absence of AIG Ma. The number of primary neurites and the number of branch points were not affected in these assays. This suggests that the AIG Ma may mediate a common activity of growth factor activated systems that regulate neurite elongation. This work was supported in part by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation.

699.6

DIFFERENTIAL EFFECTS OF MICROTUBULE INHIBITORS ON AXONAL BRANCHING AND ELONGATION OF CULTURED RAT HIPPOCAMPAL NEURONS. A. Asanuma, H. Saito and S. Aizawa, Dept. of Chem. Pharmacol., Fac. of Pharmac. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

We have previously found that basic fibroblast growth factor (bFGF) specifically enhances neurite branching without affecting neurite elongation of cultured hippocampal neurons, while astrocyte-conditioned medium (ACM) promotes the axonal elongation but not branching. This finding suggests that neurite elongation and branching are independent mechanisms which may be regulated by different factors. To further investigate these mechanisms, we have examined the effect of the microtubule inhibitors taxol and colchicine on the actions of NGF and ACM. The dissociated hippocampal neurons from 18-day-old embryos of Wistar rats were plated on polylysine-coated culture dishes at a density of 2,500 cells/cm². After incubation in serum-containing medium for 24 hr, the medium was changed to serum-free medium. The cells were cultured for 24 hr, and then neurite bearing processes were randomly selected and photographed before addition of drugs. The same cell was photographed 24 and 48 hrs after addition of drugs, and changes in morphological parameters were compared. Taxol (50 nM) alone did not affect the morphology of neurons cultured under control conditions, but significantly reduced the length of the axonal elongation was not affected by the same concentration of taxol. Colchicine (10 nM) showed similar effects as taxol. These results suggest that microtubules play more important roles in the axonal branching than in the axonal elongation.

699.7


Extensive remodeling of neuronal processes during metamorphosis is a prominent feature of nervous system development in the moth, Manduca sexta. Leg motor neurons (MN, MNb) which are retracted during metamorphosis, undergo a severe dendritic regression at the end of larval life, then grow a new, complex dendritic arbor during metamorphosis. This restructuring is controlled by the steroid molting hormone 20-hydroxyecdysone (20HE). Previous work in our laboratory has shown that leg MNs maintained in cell culture respond to 20HE exposure by growing more elaborate neuritic arbors. In order to define the cellular mechanisms underlying this morphological effect, we are comparing specific characteristics of neuritic branching patterns, and growth cone complexity and cytoskeletal structure, in leg MNs cultured with and without 20HE. Morphometric data suggest that 20HE induces a dramatic increase predominantly in higher-order neurite branching. Neurites display typical complements of microtubules and filamentous actin: microtubules are concentrated in a central region and filamentous actin accumulates in the growth cone periphery, in numerous microspikes and small filopodia. Lamellipodia are almost never observed; large flattened areas are common but are heavily invested with microtubules. Growth cones are more highly branched in the presence of 20HE, and display significantly more microspikes and filopodia as revealed by fluorescent staining of actin filaments. These results suggest that 20HE induces increased morphological complexity in cultured leg MNs by enhancing higher-order branching, perhaps via an effect on growth cone complexity and motile activity. Supported by NIH grant NS28495.

699.8
cDNA CLONING AND EXPRESSION OF XEFILIN, A NOVEL NEURONAL INTERMEDIATE FILAMENT PROTEIN IN XENOPUS LAEVIS. J. Zhou and B.L. Szaro, Dept. of Biology, SUNY-Albany, Albany, NY 12222. During axonal development and regeneration, the expression and post-translational modification of neuronal intermediate filament (NIF) proteins are cell-type specific and highly regulated. The changing molecular composition of the neurofilaments may help to regulate axonal stability at different stages of growth and to influence the caliber of diverse types of axons. Consequently, it has been suggested that NIF compositions play an important role in the growth of neurons and for modification. Consistent with this idea, fish and amphibians have unique NIF proteins whose patterns of expression suggest they may have on these animals a higher capacity for plasticity and regeneration. We report here the isolation of full length cDNA clones that encode a novel Xenopus NIF protein with a predicted molecular weight of 55.9 kDa, which we have named xefilin. Xefilin shared high amino acid sequence identity both with goldfish xefilin (overall: 66%, head: 59%, rod: 76%, tail: 30%) and with rat ixefilin (overall: 84%, head: 61%, rod: 71%, tail: 45%), but the pattern of expression was unique. Unlike grilin, which is predominantly found in the retina, xefilin mRNA was expressed throughout both the CNS and the PNS, as demonstrated in juvenile frogs by Northern blots and in situ hybridization. In preliminary analyses by Northern blot, xefilin mRNA was detected later than that of Xefinpon-NF. This differs from o-ixefilin, which appears before NF-L during mammalian development. Further characterization of xefilin expression during Xenopus neural development and optic nerve regeneration and alteration of its expression in the embryo should provide an additional focus for understanding how modifications of the neuronal cytoskeleton affect axon growth and development. Supported by NINDS grant R29 NS30682.

699.9

DEVELOPMENT, SUBCELLULAR LOCALIZATION AND REGULATION OF ALPHA INTERMIXIN IN HIPPOCAMPAL NEURONS. D.L. Roncaon* and J.L. Brown, Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, New York 10029

The differential distribution and organization of cytoskeletal elements is the basis for many of the morphological and functional differences between axons and dendrites. Alpha intermixin is a neuronal intermediate filament protein which is expressed at highest levels in the CNS during development, but can be downregulated by a variety of reagents. Immunocytochemical localization of α-intermixin in cultured hippocampal neurons reveals the intermediate filament in all neurons and axons as well as dendrites. Coincident with the developmental time of dendritic maturation and synaptogenesis, α-intermixin became concentrated in axons where long filaments could be observed. Labeling in dendritic shafts was much fainter and fuzzier, and was also present within dendritic spines–some of which could be labeled simultaneously with antibodies against glutamate receptors 2/3. Labelled spines were not observed on neurons with a GABAergic morphology. In perfused tissue sections of adult rat hippocampus, α-intermixin appeared to predominate in dendrites rather than axons. Electron microscopy revealed ultrastructural dendritic spines and associated with microtubules in dendritic shafts. Only a small population of axons was labelled. Following entorhinal cortex lesions α-intermixin immunolabeling increased in regions undergoing synaptogenesis. Although it remains to be determined whether the increase was pre- or postsynaptic, it appears that α-intermixin expression is positively correlated with synaptogenesis and may also be involved in the generation or maintenance of dendritic spine structure. Supported by NSF grant IBN-9419590.

699.10

EVIDENCE FOR A NEURONAL MYOSIN LIGHT CHAIN KINASE: cDNA CLONING AND LOCALIZATION IN GOLDFISH. X. Jian*, B.G. Storo and T. Schmidt, Dept. of Biol. Sci., SUNY-Albany, Albany, NY 12222. The control of actin-myosin interaction, which has been implicated in growth cone motility and neurite outgrowth, is regulated in non-skeletal muscle cells by myosin light chain kinase (MLCK). Pharmacological reagents specific for MLCK alter neuritic outgrowth of cultured goldfish retinal ganglion cells (Jian et al., Neuroscience 20:1311, 1995). We investigated the directed expression of cDNA clones isolated from a goldfish brain and peripheral nerve cDNA library. The cDNA clones encoded three isoforms that all shared highest sequence similarity with known smooth muscle and non-muscle MLCK's from various avian and mammalian species. Partial amino acid sequences for all three clones were compared with that of CF-MLCK. Over the regions encoding the conserved catalytic and calmodulin regulatory domains of the kinase they all shared 78% identity at the nucleotide level with the goldfish isoforms. The experiment performed with a cDNA probe derived from one of the clones revealed specific hybridization with message in several regions of the goldfish brain, most abundantly in neurons of the peripheral layer of the retina. Current efforts are directed at finishing the characterization of this cDNA and that of the other goldfish MLCK homologues. The molecular identification of goldfish neuronal MLCK homologues will form the basis for our future studies on myosin interactions in neural development and plasticity. Supported by NIH grants EY03736 to J. Schmidt and NS30682 to B. Storo.
THE FORWARD MOVEMENT OF GROWTH-ASSOCIATED ‘WAVES’ ALONG THE AXONS OF CULTURED HIPPOCAMPAL NEURONS REQUIRES INACT ACTIN FILAMENTS. G. Ruhfel* and G. Basker. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

We have previously described growth-cone-like structures referred to as ‘waves’ which periodically form at the base of axons in hippocampal cultures and travel outward along the axon to the tip, where their arrival is correlated with a spurt of growth (Ruhfel and Basker, 1990, Soc. Neurosci. Abstr.). Like growth cones, waves extend lamellipodia that are rich in filamentous actin. We wished to test whether maintenance of the wave structure was dependent on Factin and, if so, whether the wave could reform after disruption. Furthermore, we were interested in where waves reform, since reformation at the point of collapse would indicate that F-actin was required for the translocation of a wave whereas reformation at a point further along the axon would indicate that a wave could continue its forward movement independent of its Factin structure. In order to disrupt actin filaments, we treated one-day-old cultured cells with 0.25-0.5 μg/ml cytochalasin B for 10-15 min. Waves immediately ceased their forward movement as they collapsed, forming an elongated region on the axon or disappearing entirely. Although in some cases the halted wave did not re-emerge after removal of cytochalasin, in many cases the wave reformed and resumed its forward movement from the point where it had stopped. The reformation of waves after disruption by cytochalasin indicates that the capacity for forming a wave can persist even when the actin network that gives the wave its structure is disrupted. The lack of forward movement in the presence of cytochalasin leads us to conclude that intact actin filaments are necessary for the forward locomotion of waves along the axon.

Supported by NIH grants NS1712 and HD0793.

INVESTIGATION OF MAP2C TARGETING IN DROSOPHILA NEURONS BY ELECTROIC EXPRESSION. D. Adam and A. Matus. Friedrich Miescher Institute, Basel, Switzerland CH-4052.

Microtubule-associated protein 2 (MAP2) has a polarized distribution in neurons, being detectable in cell bodies and dendrites but not in axons. Tau, a related microtubule-associated protein shows the opposite pattern, being located primarily in axons. To study the molecular mechanism by which MAP2 is sorted in neurons we investigated the distribution of MAP2c, a smaller, alternatively spliced embryonic form of MAP2, in Drosophila neurons by electroic expression. Although Drosophila neurons contain no MAP2 or tau protein, mammalian MAP2c can nevertheless bind to Drosophila microtubules in vitro and in vivo (Adam et al. Neurosci. 51: 221, 1992). The GAL-UAS system was used to express MAP2c and two truncated forms of MAP2c, CT1 and AT6. CT1 contains the calaxyl-terminal region of MAP2c including the microtubule-binding domain and AT6 contains the remaining amino-terminal segment. These polypeptides were expressed in the mushroom bodies of Drosophila brain using an expression-specific GAL4 enhancer-trap line. MAP2c was most concentrated in the cell bodies and dendrites of mushroom body neurons, while the tau protein concentration was higher in axons although also detectable in cell bodies. CT1 was distributed evenly over the extent of mushroom body neurons. These Drosophila lines will be valuable for elucidating the mechanism of MAP2c sorting in the neuronal cytoplasm.

ASSOCIATION OF MITOGEN-ACTIVATED PROTEIN (MAP) KINASE WITH MICROTUBULES. M. Moribushi, Kayaviyama, J. Tani*, T. Yamasaki, J. Otani*, M. Ohara, and C. Kato. Center for Neurological Diseases, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115.

MAP kinase is a key downstream molecule in several signaling pathways. Although in vivo cytoplastic substrates of MAP kinase are unknown, the neuronal microtubule-associated proteins (MAPs) are putative substrates for this kinase. In rat primary hippocampal neurons, anti-both nucleoli and cytoplasm. Double staining of the detergent-extracted showed co-localization of MAP kinase with microtubules. Treatment of cells with microtubule-disrupting agents like nocodazole, rotenone, and MAP kinase staining on microtubules, suggesting that at least a part of the MAP kinase pool is associated with drug-stable microtubules. The association of MAP kinase with microtubules was also determined biochemically. MAP kinase was co-purified with microtubules by both temperature-dependent polymerization depolymerization cycles and the taxol method. Quantitative analysis indicated that 4% of MAP kinase in cytosolic extract is associated with microtubules. A fraction of MAP kinase was co-immunoprecipitated with MAP2c, which is a good in vitro substrate for MAP kinase, and which can be fractionated over a phospho-PAGE column and a gel filtration column to assess the co-elution of MAP kinase and MAP2c. Although MAP kinase is present in both the tubulin and MAP2c fraction of ERK2 and ERK2 in these fractions differs. This result may reflect a functional distinction between ERK1 and ERK2.

DIFFERENTIAL MICROTUBULE AND ACTIN ORGANIZATION IN CELLS TRANSFECTED WITH MAP2C AND MAP2D. M. Khorostchitskiy, L. Fehat, A. Represa, B. Bernard, G. Charbon, and C. Ben-Ari. Université René Descartes, Paris V, Unité INSERM 29, 125 Bd de Port Royal, 75014 Paris France.

High and low molecular weight forms of MAP2 proteins, MAP2A, MAP2B, and MAP2C-MAP2D are expressed in the developing brain with different ratios in the embryonic and postnatal rat brain. MAP2c is predominantly expressed in the immature brain. MAP2 variants are encoded by distinct mRNA's of 9 and 6 kb, both transcribed from the same gene. The 9 kb transcript comprises a 93 bp insertion encoding 31 additional amino acids in the cytoskeletal domain when compared to MAP2c (Fehat et al., Int. Neurochem. 25 pp 327-338, 1991). At the mRNA level show that splicing of the additional exon is regulated during ontogeny. MAP2c mRNAs being predominantly expressed over MAP2d mRNAs in all structures of the adult rat brain. We have used transient transfections in Human Embryonic Kidney cells (HEK 293) to assess the respective roles of MAP2c and MAP2d in microtubule and cytoskeletal organization, using antibodies directed against MAP2, tubulin, and actin. Our results clearly demonstrate that both microtubules and actin filaments are differently organized in the presence of MAP2c and MAP2d. Microtubule bundles are thicker in the presence of MAP2c when compared to MAP2d. A similar result is obtained with actin filaments. These proteins also demonstrate different roles when cytoskeletal components are destabilized with Nocodazole or Cytochalasin D. MAP2c and MAP2d both protect microtubules from Nocodazole destabilization. In the presence of Cytochalasin D, MAP2d, but not MAP2c, protects actin filaments from destabilization.


Microtubule associated protein, MAP1B, is a major structural component of growing neurites and is expressed at high levels in the parallel fibers of cerebellar granule cell development. To assess the role of MAP1B in neurite elongation, a 460bp fragment of MAP1B cDNA was introduced into a retroviral vector (provided by L. Lillien) in the sense or antisense orientation. After transfection of the producer cells, clones were selected which produced high titers of MAP1B sense or antisense viruses. Cerebellar granule cells were purified from neonatal rats and cultured as reaggregates in which they continue to divide. Dividing granule cells were infected with retroviruses containing either the antisense or sense MAP1B sequences. Cells were then dissociated, replated, and cultured for 48 hours. The infected cells were visualized by immunostaining for β-galactosidase coded by the retroviral reporter gene, and was detected via immunofluorescence. Although infection with MAP1B antisense retrovirus did not abolish MAP1B immunoreactivity, quantitative analysis of infected (β-gal positive) granule cells showed a significant decrease in the number of cells with no neurites and in mean neurite length in cells infected with antisense MAP1B compared to those infected with sense MAP1B. Studies of in vivo injection of dividing cerebellar granule cells are now in progress. Supported by NS 24707, NS09486, NS33214, and NS24725.


Tau is a microtubule associated protein that plays a role in the organization of microtubules and axon outgrowth. Various isoforms of tau differ in the amino acids expressed during nervous system development as the result of alternative splicing and post-translational modifications of a single tau gene. Isoforms of high molecular weight (HMW) isoforms of 110-120K. A cDNA encoding the complete 4x exon specific for HMW tau was cloned by RT-PCR into an expression vector and specific polyclonal antibodies were generated against the fusion protein and the recombinant tau protein, respectively. The distribution of HMW tau in the developing and adult nervous system was studied using immunocytochemistry and double staining immunofluorescence with the HMW tau polyclonal antibodies and monoclonal antibodies that recognize all isoforms of tau. During development, tau was expressed at all ages and was also expressed in the spinal cord. During development, the expression was restricted to small and medium, but not large DRG neurons and processes after embryonic day 18. After peripheral nerve lesion, distribution of tau within the DRG remained unchanged. In the spinal cord, HMW tau was present in neurons at postnatal day 2 and attained its adult pattern in cell bodies, axons and dendrites of ventral motor neurons by day 14. In the adult rat CNS, almost all neurons that extend processes into the PNS expressed HMW tau, including all central nervous system and peripheral processes. Most sensory ganglia, the most tipically neurons of the oral, vestibular, and spiral ganglion did not express HMW tau. Retinal ganglion cells were the only CNS neuron, whose processes remain entirely within the CNS, that expressed high levels of HMW tau. The limit and specific distribution of HMW tau in these neurons may be regulated by growth-arrested signals from the periphery to the structural requirements of these axons. Supported by VA Med. Res. Service, NIH NS24707, NS24725, NS70877, and NS09486.
699.17
Although several attempts have been made, the molecular and cellular mechanisms responsible for the differential expression of various tau isoforms as well as their functional roles, PHLF formation, and neurodegeneration have not been completely clarified.
We have performed a series of studies on SYSY neuroblastoma cells differentiated by retinoic acid treatment. We found that indifferently expressed cells express predominantly the fetal isoform of tau, although at very low level. After retinoic acid treatment, mRNA species encoding either the fetal and the mature tau isoforms can be detected. The fetal tau isoform appeared to be more expressed than the mature one. These cells were found resistant to glutamic acid treatment and sensitive to the neurotoxicity induced by doxorubicine (10 nM). The neurotoxic effects elicited by doxorubicine was temporally correlated with an increased of tau immunoreactivity, as measured by Alz50 antibody.
Finally, immunocytochemistry and Western analysis showed tau protein in the nucleus. The data suggest that tau is more than a microtubule associated protein, with possible role in cell differentiation/dysdifferentiation processes.

699.18
MICROTUBULE REORGANISATION IS OBLIGATORY FOR GROWTH CONE TURNING. T. Williamson, P.R. Gordon-Weeks*, M. Schachner1 and J. Triller1, D.B.R.C., King's College London, 26-29 Drury Lane, London WC2B 7SR. 1Department of Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland.
We have shown previously that growth cones of chick dorsal root ganglion cells turn at sharp substrate borders between the extracellular matrix molecules laminin and laminin or in vivo and grow along the laminin side of the border. To gain insight into the role of microtubules in growth cone turning, we have compared the organisation of microtubules in growth cones advancing on uniform laminin substrates with their organisation in growth cones that have encountered a laminin-tensin border. Further evidence for the involvement of microtubule rearrangement in growth cone turning was provided by experiments in which growth cones approached tensin borders in the presence of substoichiometric concentrations of the microtubule stabilising compound, taxol. Taxol altered the organisation of microtubules in growth cones growing on laminin by restricting their distribution to the proximal regions of the growth cone and increasing their bundling. Taxol did not stop growth cone advance on laminin. When growing in the presence of taxol, growth cones at tensin borders were not able to turn and grow along the laminin-tensin border, and consequently stopped at the border. Growth cones were arrested at borders for as long as taxol was present (up to six hours in these experiments) without showing any signs of drug toxicity. These effects of taxol were reversible. Together, these results suggest that microtubules reorganisation in growth cones is a necessary event in growth cone turning.

699.19
Axons and growth cones possess a spectrin/actin based network closely associated with the inner side of the cell membrane, the cortical cytoskeleton. This structure serves diverse functions during axonal outgrowth, such as surface shaping, modulation of integral membrane proteins, and signal transduction. We developed two strategies to prepare material enriched for neural cortical cytoskeleton from embryonic chick nervous tissue. The first strategy combined the isolation of a membrane/cortical cytoskeleton fraction by density gradient centrifugation with an enzymatic degradation of cell surface proteins. The second strategy is based on the attachment of marker molecules. Monoclonal antibodies (mabs) generated using both preparations as immunization material were screened for recognition of subcellular structures in axons and growth cones of retinal neurons in culture. A proportion of the mabs bind to structures in the growth cone periphery, a cell region composed almost exclusively of cell membrane and cytoskeleton, and are likely to recognize components of the cortical cytoskeleton. Expression patterns and the apparent molecular weights of the detected proteins indicate that a subset of the mabs is directed against known components, whereas others appear to detect novel components of the cortical cytoskeleton. We are currently immunopurifying and sequencing a number of proteins defined by the mabs.

FORMATION AND SPECIFICITY OF SYNAPESES: AGRIN

700.1
The clustering of acetylcholine receptors (AChRs) to high densities beneath presynaptic active zones, which is the hallmark of postsynaptic specialisation at the neuromuscular junction (NMJ), appears likely to be mediated in part by the extracellular domain of agrin. This has been demonstrated by observations that 1) application of certain soluble agrin isoforms to cultured muscle cells induces the formation of numerous AChR clusters in the muscle membrane, 2) agrin is highly enriched at neuromuscular synapses, and 3) certain anti-agrin antibodies appear to block NMJ formation in culture. However, our previous studies have also suggested the role of endogenous, muscle-bound growth factors in forming postsynaptic specialisations. Here we have coated agarose-coated beads with purified, full-length agrin isoforms (100ng/ml) to compare their ability to locally induce AChR clustering to that of growth factor-coated beads, when applied to Krebs muscle cell cultures. In addition we incubated agrin-coated beads conditionally with the heparin-binding growth factor HB-GAM to look for agrin-growth factor interactions. Here we report that locally applied, agrin-coated beads cause AChR clustering at ~25% of bead muscle contact, (BMC). However, agrin beads further incubated with HB-GAM, bind this factor as shown by immunocytophochy and cause AChR clustering at ~80% of BMC's. Beads coated similarly with BSA and HB-GAM have no effect. This may mediate AChR clustering by interacting with muscle bound growth factors, stimulating intracellular signal transduction pathways leading to AChR redistribution. (Supported by NIH and MDA)

700.2
AXONAL TARGETING OF AGRIN IN CULTURED RAT DORSAL HORN NEURONS: A. Fischer², C. Bechade³ and A. Müller² Institute of Anatomy, Bregen 9, 1005 Lausanne, Switzerland. ²Ecole Normale Supérieure, INSERM CIF 9410, 75005 Paris, France.
Agrin transcripts are broadly expressed in the CNS, including in non-cholinergic areas. The role of agrin in motoneurons, but not in other neurons, is well established. To approach this question, the presence and cellular distribution of agrin in non-motoneurons was analyzed in primary cultures of dorsal horn neurons, dissociated at E14 and kept in vitro for 1 to 7 days. We previously showed that growths make up less than 1% of all cells.
We now show : 1) the expression of the 4 agrin isoforms B0, B8, B11 and B19 (see Ruegg et al., 1992) by RT-PCR; 2) their developmental regulation during neuronal maturation, B0 being expressed first, then - during the period of synaptogenesis, the active isoforms (B20) are preferentially expressed; 3) that agrin-like immunoreactivity can be detected by Western blots both in the supernatant and in the cells; 4) using double immunostaining with anti-agrin antibodies (recognizing all isoforms) and anti-MAP2 or anti-Tau 1 antibodies respectively, an enrichment of agrin labeling in all axons. These data indicate that in our cultures, agrin is synthesized by almost all neurons and is targeted to axons. These observations suggest that, as in the neuromuscular junction, agrin plays a key role in pre- to postsynaptic interactions. Supported by Swiss NSF 31-39713.93, AFM and IRME grants.
000.3 ALTERNATIVE mRNA SPlicing OF AGRIN REGULATES BINDING TO HEPARIN AND α-DYSTROGLYCAN. M. Geisemann, A. J. Denner, Y. Cavalli, A. Brandt, P. Schumacher, U. W. Adamson, and M. A. Ruegg. Dept. of Pharmacology and Biophysics, Biozentrum, University of Basel, CH-4056 Basel, Switzerland.

Agrin is a bivalent acidic proteoglycan with an apparent molecular weight of more than 400 kD that induces the aggregation of acetylcholine receptors (AChRs) on cultured myotubes. Several isoforms are generated as a result of alternative mRNA splicing. While the chick agrin transcript (4 amino acid (aa) inserted at site A; 8 aa inserted at site B, both located near the C-terminal end) induces agrin aggregation on myotubes at picomolar concentrations, agrin transcripts (no insert at both sites) is inactive. A 45 kD C-terminal fragment of agrin(257-654) that contains inserts at both sites A and B retains high activity. A 21 kD C-terminal fragment (c21ag) is still active, but only at 100-fold higher concentrations.

000.4 CHARACTERIZATION OF FULL-LENGTH CHICK AGRIN, A BASAL LAMINA PROTEIN INVOLVED IN THE FORMATION OF SYNAPSES. A. J. Denner, M. Geisemann, R. Schumacher, and M. A. Ruegg. Dept. of Pharmacology and Biophysics, Biozentrum, University of Basel, 4056 Basel, Switzerland.

Agrin is a protein of the extracellular matrix with a calculated molecular weight of 220 kD that induces the aggregation of acetylcholine receptors (AChRs) and other molecules concentrated at the neuromuscular synapse. A 45 kD C-terminal fragment of agrin is sufficient for this activity. To investigate functional properties of the agrin isoforms, we truncated the 100 amino acid (aa) isoforms described full-length chick cDNA into COI cells. However, recombinant agrin was not secreted from cells. Here we describe a 5′ extended construct, probably encoding complete agrin, that is secreted from CHO cells. The novel sequences were found by primer extension studies and isolation of the agrin gene.

They extend the coding sequence at the 5′ end by ~300 bp. Protein sequence encoded by this extension is highly homologous to a 15 amino acid peptide of a heparan sulfate proteoglycan (HSPG) isolated from bovine kidney (Hagen et al. 1993 JBC 268, 7261). Recombinant agrin is active in inducing AChR aggregation depending on the splice variant. It has an apparent molecular weight between 400 and 600 kD and carries glycosaminoglycan side chains attached to the N-terminal part that are characteristic for a HSPG. Polyclonal antisera against agrin detects agrin-like protein in tissue homogenates that also has an apparent molecular weight between 400 and 600 kD. In addition, we have found a novel site of 7 amino acids at the N-terminal part that is alternatively spliced. While motor neurons of 5 day old chick embryos synthesize agrin mRNA encording the 7 amino acid insert, muscle cells predominantly express transcripts that lack this insert. These experiments will allow us to investigate binding properties of recombinant agrin to possibly get insights into other functions of this molecule.


Sodium channels and ACh receptors are expressed at particularly high concentrations at the neuromuscular junction. Agrin is known to play a key role in the aggregation of AChR receptors. Five isoforms of agrin are known to be differentially expressed at different developmental stages as well as by different tissues. The isoforms of agrin also differ in their ability to aggregate ACh receptors. Lupa and Caldwell (J. Biol. Chem., 1991, 115765) have shown that Torpedo agrin presents in the media of rat muscle fibular cultures was unable to cluster sodium channels. The inability of agrin to cluster sodium channels in that study may have been the result of presenting the wrong isoform of agrin to the cells in the study. We are testing the ability of different isoforms of agrin to aggregate sodium channels. Adult rat muscle fibers from the flexor digitorum brevis are dissociated and co-cultured with CHO cells that have been transfected with various isoforms of agrin and express it on the extrasynaptic side of the membrane (Ferns et al., 1993, JCB, 811079). Sodium channels are detected with immunocytochemical techniques using a polyclonal antibody to sodium channels. CHO cells expressing the A488 isoform of agrin cause clustering of sodium channels when attached to the perijunctional and near-perijunctional regions of the fibers after one day in culture. CHO cells attached more distally do not cause aggregation at a level detectable by this process. Wild type CHO cells do not cause aggregation of sodium channels. We are also testing the ability of CHO cells expressing the A419, A411 and A410 to aggregate sodium channels.

000.6 AGRIN FROM NG108-15 INDUCES ACETYLCHOLINE RECEPTOR AGGREGATION ON PRIMARY RAT MYOTUBE CULTURES. S. Dun, R. S. K. Sc and K. W. Tsim*. Dept. of Pharmacology, The Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, Hong Kong.

Agrin, a protein isolated from the synaptic basal lamina, directs the formation of the postsynaptic apparatus on developing and regenerating neuromuscular junctions. The full-length cDNAs for agrin have been cloned in chick and rat species; both are ~8 kb in size and encode proteins with a deduced molecular weight of over 200 kDa. The expression of agrin is not restricted to the motor neurons of spinal cord; it is also expressed in other tissues including the brain. We study the expression of agrin and its isoforms in a neuroblastoma cell line (NG108-15). These cells have the ability to induce the aggregation of acetylcholine receptors (AChRs) on the cultured myotubes. The co-culture of NG108-15 cells and primary rat myotubes resulted in ~10-fold increase in the AChR aggregates in the NG108-15 cultures in the surface of myotubes. Using an agrin-specific antibody, the agrin protein of ~250 kDa was identified in NG108-15 cells by immunoblotting. Our corresponding transcript (~8 kb in size) was detected by Northern blot analysis using agrin cDNA as a probe. Expression of no agrin insert at z site of agrin mRNA in NG108-15 cells was also demonstrated using polymerase chain reaction. To demonstrate the aggregation of AChRs is due to the secreted agrin from NG108-15 cells, antisense construct of agrin cDNA was transfected into the NG108-15 cells. The NG108-15 cells induced-AChR aggregation on cultured rat myotubes was blocked by such antisense cDNA transfection. These studies provide a direct evidence that the NG108-15 cell-induced AChR aggregation on cultured primary rat myotubes is mediated by agrin.

000.7 DOMAINS OF AGRIN THAT INDUCE ACHR PHOSPHORYLATION. T. Meier, M. Geisemann, V. Cavalli, M. A. Ruegg, and U. W. Adamson. Dept. of Immunology, Univ. Col. Hlth. Sci. Ctr., Denver, CO 80262 and Dept. of Pharmacology, Biozentrum, Univ. of Basel, CH-4056 Basel, Switzerland.

Differentiation of the postsynaptic apparatus at developing and regenerating vertebrate skeletal neuromuscular junctions is triggered by agrin. Agrin is a 400-600 kD heparan sulfate proteoglycan with several regions known to induce aggregation in other extracellular matrix proteins. In addition, several isoforms of agrin have been identified that arise by alternative splicing of a single agrin gene. When added to intact cultures, agrin induces formation of specializations at which acetylcholine receptors (AChRs) and other components of the postsynaptic apparatus accumulate, and also induces tyrosine phosphorylation of AChRs. Treatment with block agrin-induced protein tyrosine phosphorylation prevents agrin aggregation, suggesting that phosphorylation may play a role in receptor aggregation. In culture, agrin binds to α-dystroglycan and it has been proposed that this binding mediates AChR aggregation. To test further the relationship between tyrosine phosphorylation and receptor aggregation, we have compared the ability of fragments of various agrin isoforms to trigger AChR aggregation in culture. Agrin-induced tyrosine phosphorylation is required for AChR aggregation, and also cause AChR tyrosine phosphorylation, while domains that mediate binding of agrin to α-dystroglycan are not required for either AChR aggregation or phosphorylation.
REGULATION OF AGRIN GENE EXPRESSION DURING DEVELOPMENT OF MOUSE THALAMUS AND SOMATOSENSORY CORTEX. Z. Lu1, J.L. Hasselbacher1,2, D.E. O'Neal1,2, and M.A. Smith1,2. 1Dept. of Anat. and Neurobiol. and 2Cell Biol., University of California at Irvine, Irvine, CA 92717.

Agrin mRNA is expressed by many neuronal populations in developing and adult rat brain, suggesting a role for agrin in the formation and maintenance of synapses in the mammalian CNS. To investigate this possibility we have performed a detailed study of agrin gene expression in the mouse thalamocortical system during the first 3 weeks of postnatal development, a period when functional connections are formed between thalamus and cortex as well as within cortex form and mature. Whereas all four agrin mRNAs resulting from alternative splicing at the 2' region are present in RNA from ventrobasal thalamic, the relative abundance changed postnatally. Agrin mRNA4 was strongly expressed in thalamus at birth (P0) to P20. Agrin transcripts were first detected at P5, with maximal levels (>10%) occurring around P16. Agrin mRNA1 was present only during P0-P4 with a peak (>4%) at P1. Agrin19 transcripts represented about 80% of total agrin mRNA at P2. A similar pattern of agrin mRNA expression was also observed in somatosensory cortex. In parallel studies in vitro, we observed that functional connections formed between dissociated P0 cortical neurons during the first two weeks in culture and that transient changes in agrin 11, 19 and -19 mRNAs were similar to those between P0-P14 in vivo. Thus, developmental changes in agrin gene expression in vivo and in vitro are correlated with formation of functional synaptic connections, consistent with a role for agrin in synaptogenesis in the mammalian CNS. Supported by NS07351 to ZL, NS30213 to MAS and NS30109 to DOD.

IDENTIFICATION OF A NOVEL AGRIN ISOFORM PREFERENTIALLY EXPRESSED IN NON-NEURONAL CELLS. G. Tesfay1, A. Nagler1, W. Halfter2, and G.L. Cole1,2. 1Neurotechnology Center and Dept. of Cell Biology, Neurobiology and Anatomy, The Ohio State Univ., Columbus, OH 43210. 2Dept. of Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

A novel isoform of agrin was identified based on the isolation of an agrin cDNA from E9 chick brain that lacks 21 bp in the N-terminal encoding region of the agrin mRNA. RT-PCR of E9 chick brain mRNA confirmed the existence of this agrin isoform in brain. Since the nolo splice variant represents only a minor fraction of agrin mRNA in brain. However, upon the analysis of chick anterior myotube smooth muscle mRNA, and cardiac muscle mRNA by RT-PCR, this novel isoform was shown to be the predominant agrin isoform in these non-neuronal cell populations. We extended our analysis to examine the expression of this agrin mRNA isoform during chick development, and showed that in brain this novel agrin isoform is up-regulated with brain development, consistent with the increase in glial number during brain development, while the agrin isoforms that do not undergo splicing and thus remains the 21 bp exon is down-regulated. We also showed that in heart both agrin isoforms are down-regulated during development. Because the 21 bp exon is inserted in the region of chick agrin which encodes the positive signal sequence of agrin, with the signal peptide cleavage site immediately preceding the positive first amino acid of the mature protein being deleted as a result of splicing, the presence or absence of this alternatively utilized exon may regulate differential processing of the agrin protein in neuronal and non-neuronal cells, respectively. Supported by NIH grant NS39891.


The prominent electrical responses visually elicited in the superficial neuropil (SN) of OC 6 mo after transplantation of a regenerating optic nerve into the tectenoccephalon (T) were evocable only from the frontal visual field, but a spatial map of the responses was not observed (Neurosci. Abs., Scala et al., '94). Transients of this type and size are recorded in the SN of the optic tectum with presentation of prey-mimetic stimuli in any sector of the visual field (Grant and Lettin, Brain Res., '91), but are never found in normal OC. Anatomical data on the sectorial origin of the ectoptic fibers was sought to investigate the basis of the frontal-field-dominance in this projection. Mixed injections of biotin-dextran (BDA) and H-amine acids were given intraventricularly immediately after cutting across a defined sector of the retinal nerve fiber layer in frogs surviving 6-8 mos after transplantation of the optic nerve into T. The injected specimens survived an additional 3 days to allow time for anterograde migration of the BDA in the cut retinal axons and transport of the H-label by the uninnjured ganglion cells into the cortical regions of the retina. BDA-labeling was examined both by LM and EM. When the cuts were made across temporal sectors of the retina, which images the frontal-field, the BDA-labeled terminal plexus in the experimental animals occupied the posteroordinal half of the ectopic projection-field. After nasal retinal lesions, the BDA-labeled terminals were distributed in the more anteroverentral part of the projection-field. The pattern of radiolabel in autoradiographs of adjacent sections was complementary to the BDA-labeling, but with significant overlap in the fields defined by the two tracers. Thus, the ectopic projection arises from both the temporal and nasal halves of the retina, but only the former becomes capable of evoking the large-amplitude responses. (Supported by NIH Grant EY02284)
**701.3 ASYMMETRIC NASAL/TEMPORAL EXPRESSION OF AN ALTERNATIVELY SPliced GENE WITHIN THE DEVELOPING RETINA.**

W.M. James, D.R. Fekete, D.J. Solkis and S.C. McLean.

Development of the topographic pattern of axonal connections from the eye to the brain is believed to involve molecules expressed asymmetrically across the developing retina that mark cell position. In an attempt to identify such molecules, differential kinetic enrichment was employed to isolate asymmetrically expressed mRNAs in the nasal-temporal axis of the embryonic chick retina. A PCR amplified cDNA representing a mRNA enriched in the nasal side of the retina was used as a screen on embryonic retinal cDNA library. Seven clones were isolated. Restriction analysis suggested that these clones share a 2kb "conserved" region, and each had a "variable" region of up to 2kb. A probe prepared from a fragment of the conserved region of one clone hybridized to all seven clones. Two separate fragments from the variable region of one clone hybridized to subsets of the seven clones. RNA blot analysis suggested that the conserved region is expressed equally on the two sides of the retina, while transcripts containing either of two fragments from the variable region were more abundant on the nasal side of embryonic retina. These results suggest the existence of an alternatively spliced gene, the products of which are asymmetrically expressed across the developing retina. Comparison of partial sequence from one clone suggested that it is a previously unreported gene. (Supported by NIH grants EY03571 and EY07133.)

**701.4 QUANTITATIVE DISTRIBUTION OF GABA-IMMUNOREACTIVE TERMINALS AFTER SENSORY DEPRIVATION IN THE RAT BARREL CORTEX.**

C. Beaulieu* and C. Crevier. Dept Pathology and CRSN, Université de Montréal, Montréal, (Qc) Canada.

We have previously shown that cerebral sensory deprivation leads to a 50% reduction in the numerical density (Nv) of synaptic contacts formed by GABA(+) boutons in layer IV of deprived barrels as compared to controls. To estimate whether this change was due to an actual loss of GABA(+)-axon terminals, we calculated their number in layer IV of the barrel cortex of rats which had their vibrissae on the right face continuously removed from birth.

This suggests that the decrease of contacts could be due not only to a loss of GABA(+) terminals but also to a decrease of the number of synaptic contacts formed by each bouton. However, it is also possible that the decrease of synaptic contacts was consequent to a preferential loss of a subpopulation of GABA(+) terminals making on average, a high number of contacts. This is currently under investigation. (Supported by MRC, FRQS, and FCAR.)

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**701.5 GABA IMMUNOCYTOCHEMICAL ANALYSIS OF CEREBELLAR EXPLANT CULTURES FROM THE LURCHER MUTANT AND WILD-TYPE MOUSE.**


Lurcher is an autosomal dominant murine mutation. Lurcher heterozygotes (+/l) lose all their cerebellar Purkinje cells by adulthood (Caddy and Biscoe, 1979, Phil. Trans. R. Soc. B. 287: 187-201). Chimeric analysis has shown that the primary target of the +/l mutation is the cerebellar Purkinje cell (Wettas et al., 1979, J. Embryo Genet. 2: 87-96). Purkinje cells from the +/l mutant mouse survive and differentiate in long-term cerebellar explant cultures. Quantitative cell counting revealed that cell death is non-autonomous (Doughty et al., 1995. J. Comp. Neurol. in press). The neuronal environment and synaptic investment of Purkinje cells in +/l and +/- (control) cerebellar explant cultures was examined using immunocytochemistry (GABA immunocytochemistry). Cerebellar explants from 2 days postnatal (P2) +/- and +/- mutant mice were grown for 15 days in vitro before being processed for GABA immunocytochemistry. GABA-immunostained explants were examined in the light and electron microscope. GABA-immunostained Golgi and basket and/or stellate cells were scattered throughout the +/- and +/- cerebellar explant cultures and there was no in vivo-like arrangement of the immunoreactive somata. Quantitative observations did not indicate any difference in the numbers and distribution of the GABA-immunostained cells in the +/- and +/- cultures. Electron microscopical analysis revealed a similar, complex distribution of the GABA-immunostained in the +/- and +/- cerebellar explant cultures. The somaata of Golgi and basket and/or stellate cells were GABA-immunostained, whereas their neurites had a varied immunoreactivity; GABA-immunostained and GABA-immunonegative axons formed synapses directly with the shafts of spiny Purkinje cell dendrites; whilst all the synapses on the dendritic spines of Purkinje cells were from GABA-immunonegative parallel fibres. (Supported by The Welcome Trust.)

**701.6 DESCRIBING AND GENERATING COMPLEX DENDRITIC MORPHOLOGIES USING L-SYSTEMS.**

D. Rosenbluth, I. Allman*

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We describe the use of L-systems for both the generation and description of complex dendritic morphologies. L-systems provide power and flexibility in describing a wide variety of neuron morphologies. We present a set of rules for describing neuron morphologies using L-systems.

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**701.7 STABILISING NEUROMUSCULAR CONTACTS INCREASES MOTONEURONE SURVIVAL AFTER NEONATAL NEUROGENIC INJURY IN RATS.**


Department of Anatomy and Developmental Biology, University College London, Gower Street, London WCIE 6BT, UK.

After a synaptic nerve crush at birth the rat soleus muscle is permanently weak. The weakness is caused a) by loss of a proportion of its motoneurones and b) by the failure of the surviving motoneurones that are successful in reaching the muscle and reinervate it 7 days later to expand their peripheral field. Here we attempted to maintain new neuromuscular contacts by applying heparin, an inhibitor of the calcium activated neutral protease. This protease appears to be involved in the loss of neuromuscular contacts during development and sprouting. This study shows that in muscles treated with heparin the reduction in weight and force after nerve crush at birth was significantly less than in those that were untreated. Moreover, the heparin treated muscles had significantly higher numbers of motor units than untreated muscles. In the heparin treated animals the number of remaining motor units in the soleus muscle was 67 ± 37 (S.E.M., n=8), whereas in the NaCl treated animals soleus had only 3 ± 0.4 (S.E.M., n=10) motor units. Thus, protecting regenerating neuromuscular junctions during early stages of reinnervation rescues motoneurones and improves muscle recovery.

**701.8 REGULATION OF N-TYPE CALCIUM CHANNEL DISTRIBUTION IN ISOLATED HIPPOCAMPAL NEURONS.**

R. Lessene*1, J. Crevier*,1, E.L. Trumpf2, R. Davies1, D.R. Whidden3, K.P. Campbell and P.L. Hruby3

1 Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011; 2 Howard Hughes Medical Institute, Dept. of Physiology and Biophysics, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The influx of calcium through voltage dependent N-type calcium channels stimulates vesicle fusion during synaptic transmission. Little is known, however, regarding the causes that determine the expression of these channels and their localization during development. For example, is neuron-neuron contact required for normal N-type channel expression, distribution and function? To address this question, hippocampal neurons were grown under conditions which resulted in either isolated neurons or neurons with neuron-neuron contacts. Differences in the expression of the N-type calcium channel subunit a_2delta were then compared using immunocytochemistry. At 4 days in culture (DIC) neurons that were allowed neuron-neuron contact displayed diffuse a_2delta immunoreactivity in the soma as well as immunonegativity concentrated in punctate structures within neuron long dendritic arborization from a_2delta at 8 DIC, but in contrast, the distribution pattern was diffuse and non-punctate in neurites. This distribution pattern was comparable to an "immature" pattern observed for 4 day neurons which were allowed neuron-neuron contact. To test this hypothesis, channel subunits observed by immunocytochemistry form functional calcium channels in neurons displaying the "immature" pattern, whole-cell patch clamp experiments were performed. By 4 DIC-sensitive calcium current was present (24.6±3.5% block, n=6). These data suggest that N-type calcium channel subunits are assembled into functional channels to be later redistributed at sites of synaptic contact in response to signals provided by target neurones.

The roles of glia in brain have been regarded as important for not only structural and nourishing support of neurons but also active modulation of local neuronal functions. In some recent reports, it is shown that glial cells have a potential to mediate messages from a postsynaptic cell to presynaptic one. To elucidate precise roles of glia in neuronal systems, a glia-free neuronal culture is a useful system. Although inhibitors of cell proliferations such as cytosine arabinoside has so far been useful, they have so far to have toxic effects on neuronal growth and survival. Therefore, we have developed a novel procedure to prepare a glia-free neuronal culture system by adding certain peptides containing a cell adhesion-relating sequence such as RGD into culture medium. The synapse-formation in the culture was found to proceed in a similar way to that in the glia-containing one. To form synaptic connection, a growth cone of an axon is thought to recognize a specific target molecule such as cell adhesion molecules. The results obtained in the culture indicate that a protein that recognizes RGD sequence is not involved in the synaptogenesis in the culture.


In explant cultures of embryonic rat trigeminal ganglion (TG) with age same whisker pad and more mature brainstem explants (accessed through the brainstem trigeminal nuclei BSTC), TG axons grow and arborize in both targets (Erzurumlu et al., 1993). Here we further characterize the morphological and cellular aspects of this connectivity. Dil-labeled trigeminal axons form small, well-defined arbors within the BSTC. Biotinylated BSTC neurons have small, irregular shaped soma, smooth dendrites, and simple dendritic branching patterns. Of interest was to examine the membrane properties of BSTC neurons and to determine whether TG input gives rise to postsynaptic responses. Intracellular recordings indicate that BSTC neurons exhibit overshooting action potentials, and at least two after hyperpolarizations following the depolarizing current injection (IC). These responses are similar to those of sensory forebrain neurons. These responses can be due to the characteristic differences in the excitability of the two neuronal populations. An additional characteristic of BSTC neurons is that they have a lower threshold for the generation of action potentials (EPSPs) and a lower time constant (τ) than TG neurons. These responses are likely related to the lower input resistance of BSTC neurons. Further characterization of these responses is needed to determine their physiological significance.

701.13 RECORDING ELECTRICAL ACTIVITY FROM HEILOSMA BUCCAL GLANDS AND CENTRAL RING GALLINDIA CULTURED ON A PLANAR MULTIELECTRODE ARRAY. I. Kim*, Y. J. Bimbo, and A. Kawana†. Dept. of Biology, University of Southwestern Louisiana, Lafayette, LA 70504, and NTT Basis Research Labs., Atsugi-shi, Kanagawa,244-01 Japan.

Recent advances in techniques for multielectrode recording and stimulation of multiple extracellular microelectrodes allow simultaneous and chronic observation of electrophysiological events in populations of neurons. By using the planar multi-electrode array (PEA) system, the long term profile of electrical activity has been recorded from electrophysiologically cultured rat cortical networks, and the periodical electrical stimulation through electrodes induced bursting response in silent cultures of rat cortical neurons (A. Kawana et al., Soc. Neurosci., 1992). The merits of simultaneous chronic multi-site recording and stimulation are utilized to study dynamics of functional connectivity in cultured Hela/Summa neural networks. In small Hela/Summa, several observations suggested that patterned neuronal activity in the buccal ganglia (BG) for feeding behavior involves descending excitatory and inhibitory afferents from the central ring ganglion (CRG: 9 pairs of ganglion). In order to study the dynamics of functional connectivity and electrical coupling between buccal pattern generation in BG and each ganglion of CRG, the neural network among BG and each ganglion of CRG was generated on a planar orthogonally-patterned array of 64 electrodes. The electrodes (each size: 50x50 µm) were separated by 350 µm distance (Y. J. Bimbo et al., IEEE Trans. BME). Isolated BG and CRG were tyraminized and ganglionic connective tissues were desheathed with an electrically sharpened microknife. The desheathed masses of BG and CRG were placed and cultured for the formation of neural networks on the PEA. Each mass of ganglion covered around 4-16 electrodes, and the detected amplitude of electrical activity were ranged around 40 µV. The dynamical changes of electrical activity of the desheathed mass of ganglion were simultaneously recorded from multiple electrodes, and analyzed.


Exposure to phencyclidine (PCP) during development often produces detrimental effects including long lasting alterations in motor function. PCP is known to be a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor. We have previously shown that embryonic exposure to acute PCP exposure to the NMDA receptor antagonist, MK-801, disrupts the development of cutaneous nerve projections in the chick spinal cord (Dev. Neurosci., 16, 163-180, 1994). However, these effects only occur when PCP is applied in spinal cord development, chick embryos were exposed to PCP during the period when primary sensory afferents form connections in the spinal cord. Groups of chick embryos treated daily with either 1.0 or 10.0 mg/kg of PCP in sterile Tyrode's solution (physiological saline, ST). Control embryos were treated with equivalent volumes of ST. Applications of PCP or ST began in embryonic day 5 (E5) and continued until the animals were sacrificed at E14. Embryonic motility was assessed daily at 5 time points: 15 min before drug exposure, and 15 min, 30 min, 1 h, and 2 h after drug exposure. Following sacrifice of the animals at E14, the pattern of cutaneous nerve projections in the dorsal horn was determined by applying fluorescent tracers to identified cutaneous nerves. PCP-treatment induced a dramatic alteration in the organization of cutaneous nerve projections. The central projection formed by the lateral femoral cutaneous nerve, which normally occupies only the ventral portion of lamina 2 in lumbarosacral segment 1, spread to occupy almost the entire cross sectional area of lamina 2. These data suggest that some of the detrimental effects of embryonic PCP exposure are due to inappropriate synapse formation by primary sensory afferents in the spinal cord dorsal horn. (Supported by AA08025).


Cajal-Retzius cells are the most typical representatives of the marginal zone-layer 1 in the neocortex and hippocampus. These transient neurons disappear during development, and their functions remain to be elucidated. Taking advantage of calcium-immunochemistry, a marker for Cajal-Retzius cells in the murine cortex (Del Rio et al., Central Cortex, 5;13), we investigate here the features of these cells in slice cultures and in vivo", and address their developmental roles. Whereas Cajal-Retzius cells in cultures taken from P0-P2 neocortex and hippocampus cell degeneration with a temporal sequence similar to that "in vivo", cells in the hippocampus persist after long incubation periods. Since Cajal-Retzius cells in the hippocampus may be involved in the attraction and targetting of developing efferent afferents (Sugup and Soriano, J. Comp. Neurol., 346,101) we have analyzed the development of the entorhino-hippocampal network of rats cultured on organotypic slices. Antengrade tracing of efferent afferents by biocytin show that growing axon overlap and are intermingled with Cajal-Retzius cells, establishing synaptic contacts. Further, cells in these co-cultures undergo cell degeneration shortly after the ingrowth of efferent afferents. These results suggest that Cajal-Retzius cells might act as guides for developing efferent areas, which might in turn influence the degeneration of these transient cells. Efforts are currently underway to determine whether the ablation of Cajal-Retzius cells in organotypic co-cultures alters the pattern of termination of efferent afferents.
1780

NEUROTRANSMITTER SYSTEMS AND CHANNELS III

702.1

WEDNESDAY PM

ONTOGENY OF FIRING PATTERNS OF DOPAMNE NEURONS IN SLICES.
Gessa. Dept, of Exp. Biology, Cagliary, Italy.
Bursts occurrence in vivo dopamine (DA) neurons is determined by N-Methyl-Daspartate (NMDA) tone in midbrain DA areas, while DA neurons, in slices exhibit
only a regular pacemaker pattern. However, application of NMDA and other EEAs
to slices from adult rats promptly increases the discharge frequency but fails to
modify the pacemaker pattern of these neurons. In our study, DA neurons showed a
biphasic, age-related, occurrence of bursting discharge. It increased from about 1%
at post natal day (PND) 5 to a transient peak of more than 30% at PND 18, and
rapidly decreased to zero after PND 45. In PND 15-21 slices, application of
glutamate or kainate elevated the firing rate without pattern modification. On the
contrary, NMDA (10-50 pM) activated discharge rate and also promoted burst firing
in both bursting and non bursting neurons. NMDA induced bursts were age-related,
maximal (37%) at PND 15-21, and NMDA increased the number of spikes in bursts
irrespective of age. NMDA effects were not seen in a Ca2+ free solution or in the
presence of NMDa antagonists. At PND 15-21, bath application of AP-5 reduced
spontaneous burst occurrence and firing irregularity in 11 out 19 neurons. Wholecell current-clamp recordings revealed spontaneous bursts in DA neurons at PND
15-21, superimposed on long (2-3 sec) depolarizing jumps. Low-threshold spikes,
generating bursts of action potentials, were elicited by either depolarizing, (at -65
mV), or at the offset of hyperpolarizing currents steps (at -80 mV). Using electrodes
filled with 145 mM CsCl, burst activity was paroxystically increased. In voltageclamp recordings, spontaneous miniature excitatory post-synaptic currents
(mEPSCs) were recorded in 8/12 bursting and in 1/10 pacemaker DA neurons at
PND 15-21. Addition of 100 |iM 7-C1-KA abolished the mEPSCs and produced an
outward current. Since burst firing modulates Ca2+ influx, thereby the Ca2+mediated DA release, the pharmacological manipulation of burst production might
provide new strategies for those pathologies related to DA system dysfunction,
including Parkinson's disease, abuse of drugs and schizophrenia. In addition, the
elevated NMDA sensitivity displayed by DA neurons during the critical third week
after birth might have relevance in NMDA-controlled phenomena, such as synaptic
plasticity, neuro-architecture and neurotoxicity.

702.2
ASPHYXIA DURING BIRTH INDUCES LONG-LASTING
CHANGES IN MESO-TELENCEPHALIC DOPAMINE
RECEPTORS OF THE MALE RAT.
aY. Chen, bM, Hillefors-Berglund. aB, Bielke. & CM. HerreraMarschitz. hg. von Euler and aK. Andersson*.
aDept. Internal Medicine, Karolinska Institutet, Huddinge Hospital,
141 86 Huddinge, Sweden, bDept. Neuroscience and cDept Physiology
& Pharmacology, Karolinska Institutet, Sweden.
Asphyxia was induced during birth to male Sprague-Dawley rat pups.
At an age of 4 weeks dopamine receptors were analyzed by quantitative
autoradiography in the mesencephalon (A9 and A10), the caudate
nucleus, the accumbens nucleus, and the olfactory tubercle. Mild (1516 min), as well as, severe (19-20 min) perinatal asphyxia reduced Dj
antagonist binding (pHJSCH-23390 in the presence of ketanserine) in
the accumbens nucleus, the olfactory tubercle and the A9 region, and
increased Di agonist affinity ([3H]dopamine in the presence of
raclopride) in the accumbens nucleus and the olfactory tubercle. Mild
asphyxia did not change D2 antagonist binding ([ 125I] iodosulpride) or
D2 agonist affinity ([3H]/V-propylnorapomorphine), while severe
asphyxia reduced D 2 agonist affinity in the accumbens nucleus. D3
agonist affinity ([3H]7-OH-D.PAT) was increased only following mild
asphyxia.
In conclusion, asphyxia during birth induces long-lasting changes
in dopamine receptor binding in the meso-telencephalic dopamine
systems, which may contribute to previously reported behavioral
changes. Perinatal asphyxia may thus be of importance for development
of neurodegenerative disorders.

702.3

702.4

DOPAMINE SYNTHESIS INHIBITION BY (±)-7-OH-DPAT IN STRIATUM,
ACCUMBENS, AND PREFRONTAL CORTEX IN DEVELOPING RATS. L
L. AnAnrses* *nd M, H, Teichcr, Departmern ofPsysyiatia\ HctfvrrdMMical
School, Laboratory of Developmental Psychopharmacology, McLean Hospital,
Belmont, MA 02178.
Dopamine (DA) synthesis modulation by the D3 receptor agonist (±)-7-OHDPa T was explored in striatum, accumbens, and prefrontal cortex of developing

3.6 KILOBASES OF THE 5' FLANKING DNA OF THE MOUSE TYROSINE
HYDROXYLASE GENE DIRECTS BRAIN-SPECIFIC BUT NOT
CATECHOLAMINERGIC-SPECIFIC EXPRESSION.
W.W. Morgan*.
Dept, of Cellular and Structural Biology, Univ. Texas Hlth. Sci Ctr. at San
Antonio, TX 78284-7762.
The expression of the tyrosine hydroxylase (TH) gene is regulated by very
precise molecular mechanisms which limit its activation to catecholaminei-gte
neurons located within the brain, the sympathetic chain ganglia and
paraganglia and to adrenal medullary cells. To study these processes,
approximately 3.6 kilobases (kb) of the 5' flanking DNA of the mouse TH gene
was inserted upstream of a E. coli (-galactosidase reporter (lac Z). This fusion
gene (TH3.6LAC) was introduced into one-cell embryos of C57BL/6 mice, and
transgenic mice were identified by PCR analysis. Analyses of fl-galactosidase
activity and the demonstration of TH3.6LAC mRNA by RNase protection
assays suggested that the chimeric gene was activated in every region of the
brain examined including the olfactory bulb, brainstem, hippocampus, cerebral
cortex, striatum, cerebellum and diencephalon, as well as the adrenal gland.
However, no evidence of TH3.6LAC activation was observed in the liver,
kidney, spleen, lung or thymus. Similar results were observed in different
transgenic founder lines. Histochemical analysis suggests that the reporter
gene is activated in catecaolaminergio and some selected noncatecaolaminergic nuclei of the brain and in the adrenal medulla. Therefore,
3.6 kb of the 5' flanking DNA of the mouse TH gene appears sufficient to direct
the expression of the lac Z reporter to the brain and the adrenal medulla but
not to limit this activation to catecholamine^c nuclei.
Supported by
GM43763.

rats (10 - 40 days of age) using the GBL autoreceptor model. GBL produced an
age-dependent increase in DA synthesis that was inhibited by (±)-7-OH-DPAT
(0.1 - 13.5 mg/kg) in all regions. In striatum 7-OH-DPAT exerted a greater
maxima! inhibitory effect at 10-20 days (85% inhibition) than at 30-40 days (61%
inhibition). 7-OH-DPAT inhibited DA synthesis by 66 % in the aooumbans, with
no significant change with age. In prefrontal cortex GBL increased synthesis
between 10-30 days, but had no effect at 40 days. 7-OH-DPAT inhibited DA
synthesis 60% between 10-30 days. Eticlopride antagonized the action of 7-OHDPAT in all regions, suggesting DA receptor mediation. These data confirm the
existence of synthesis-modulating autoreceptor function in prefrontal cortex that
recedes by 40 days. In all regions, the IC50 increased with age, indicating a
decrease in autoreceptor sensitivity with maturation. Curiously, the striatum was
more sensitive to the inhibitory effects of 7-OH-DPAT than the mesolimbic
below;
reg ions. (See
Tab le
IC5o
in
mg/kg)
PrefrontalCortex
Striatum
Accumbens
10 Days
«0.1
0.27 ± 0.20
«0.1
1.42 ± 0.46
15 Days
0.49 ± 0.32
0.79 ± 0.43
0.41 ± 0.14
2.97 ± 0.12
20 Days
0.95 ± 0.46
30 Days
0.44 ± 0.88
0.50 ± 0.09
3.71 ± 0.53
3.53 ± 0.64
40 Days
2.46 ± 0.25
Supported by MH-43473.

702.5
THE CREATION OF A TYROSINE HYDROXYLASE-NULL MUTATION. M,
Biol.; Dept, of Neuroscience, Tufts. Univ., Boston, MA 02111
Cells containing the catecholamines dopamine, norepinephrine, and
epinephrine are present in the central and peripheral nervous systems. Tyrosine
hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, is
persistently expressed by all catecholaminergic neurons and is transiently expressed
in some cell populations during development. The role of TH in tissues where it is
transiently expressed is not yet understood. The long term goal of the creation of a
TH-nuIl mutation is to expand our knowledge of catecholamine systems, transient
TH expression, and the putative role of catecholamines in neural development.
Tyrosine hydroxylase-null mutants were generated using homologous
recombination in embryonic stem (ES) cells. In order to disrupt the TH gene, a
polyadenylation trap targeting vector was made in which genomic sequence between
exons 6 and 8 of the mouse TH gene was replaced by a thymidine kinase-neomycin
(TK-Neo) cassette without a polyadenylation signal. This construct was transfected
by electroporation into D3 ES cells that were analyzed subsequently for the presence
of homologous recombinants. Three homologous recombinant clones were detected
among 371 neomycin-resistant clones, representing an efficiency of less than 1%.
Seven chimeras were generated from the two independent ES cell clones with
ES cell contribution estimated to range from 65-100% based on coat color. Among
the seven chimeras bred to C57B16 mice, five led to germ line transmission
producing heterozygous carriers that were used subsequently to breed the mutation
to homozygosity.
Lack of TH-null mutants in our first litters leads us to suspect that disruption
of the TH gene results in embryonic lethality in homozygous animals, confirming
NS20181 and NS22675

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Neuroscience

, Volume 21, 1995

702.6
IN LAN-1 CELL LINE, TETRAHYDROBIOPTERINE INDUCES THE SYNTHESIS
OF DOPAMINE AND REDUCES THE SEROTONIN TURNOVER. A.Zuddas*.
Child Neuropsychiatry, Dept.Neuroscience, Univ.of Cagliari, °Dept. Science Human
Communication, 2nd School of Medicine, Univ.of Naples,AIIGB-CNR, Naples, Italy.
LAN-1 is a human cell line expressing tyrosine hydroxylase, muscarinic Ml and M3
receptors, veratririne-sensitive sodium channels and they lack voltage-sensitive calcium
channel. To further ■ characterize the aminergic phenotype of these cells, we measured
the monoamine and metabolite content by HPLC, the high affinity uptake and the
veratridine-inducer release of [3H]dopamine. Immunocytochemical analysis using anti
Tyrosine Hydroxylase (TH) antibodies confirmed that several cell were TH-positive:
nevertheless, no dopamine (DA) nor DA metabolite (DOPAC and HVA) were found in
the cell omogenate. However, significant amounts of serotonin (5HT) and its
metabolite 5HIAA were measured in the cells (2.6 ±1.0 and 20.5 ±1.5 ng/mg protein
at 1 d.i.v. and 3.0±0.7 and 19.3 ±2.2 ng/mg protein at 4 d.i.v, respectively). The
presence in the culture medium of 5,6,7,8 tatrahydrobiopterine (THB, 10 mM),
cofactor for both TH and tryptophane hydroxylase, was able to activate the dopamine
syntesis: at 1 d.i.v, DA, Do Pa C and HVA contents were 8.7 ± 2.7, 1.1 ±0.1 and 0.3
± 0.1 respectively. Moreover, THB significantly decreased 5HIAA (24.9 ± 0.4 vs 8.1
± 1.5 ng/mg protein in control and THB, respectively, p<0.01). Similar results were
obtained in four-day old cultures. LAN 1 cells were also able to taken up exogenous
DA. After incubation with 50 nM [3H]DA, the specific [3H]DA uptake was
456.7+17.7 fmol/well and 1231.O+33.0 ftnol/well at 1 and 4 d.i.v. respectively. On
the other hand, in [3H]DA preloaded cells, veratridine (IOOp M) was unable to induce
dopamine release. Taken together these data indicate that LAN 1 cell express TH and
the cellular machinery for the high affinity dopamine uptake: in these cells, the
dopamine synthesis could be actvated by the presence of high concentrations of THB,
making this cell line an useful model of human diseases such as dopa-responsive
dystonia. THB is also able to reduce the serotonin turnover: this appears of particular
interest for studing the role of serotonin-dopamine interactions during normal
development and in the pathogenesis of human psychiatric disorders.



The serotonin (5-HT) innervation of the developing rat visual cortex (VC), lateral geniculate nucleus (LGN), superior colliculus (SC), lateral septum (LS) and basal forebrain (BF) was examined with light and electron microscope immunocytochemistry. Comparisons between these areas showed that: 1) the distribution pattern was established gradually in some areas (LGN, SC, VC) whereas in others (VC) it showed transient features; 2) the adult pattern of innervation was attained by the end of the third postnatal week in all areas; 3) the types of synapses formed by 5-HT varicosities and the nature of postsynaptic profiles varied according to age and area of the brain examined; and 4) in all areas, the proportion of labeled varicosities forming synapses increased from birth until the end of the second week, declined markedly in the subsequent week before increasing again at a later stage. These findings support the view that: 1) the organization of the 5-HT system is related to the postsynaptic targets and, although arising in small number of neurons in the midbrain, is highly specific in many parts of the brain; and 2) synapses formed by the serotonergic system during the first two weeks of life may be related to the involvement of 5-HT neuronal events, while those formed after the third week of life may be involved in the mediation of neurotransmitter effects by this monoamine.

POSSIBLE POPULATION DIFFERENCES IN THE EXPRESSION OF GLYCINE RECEPTOR a2 ISOFORMS BY EMBRYONIC RAT SPINAL CORD NEURONS DURING DEVELOPMENT. M.D. Withers and P.A. St.John. Program in Neuroscience, Dep. of Anat., Univ. of Arizona, Tucson, AZ 85724.

Previous results on isoform-binding assays, fluorescence microscopy with monoclonal antibodies and whole-cell patch-clamp recordings provide evidence for a change in the predominant expression of GlyR a2 subunits by embryonic spinal cord neurons from a relatively strychnine-sensitive isoform, presumed to be a2*. One or more strychnine-sensitive isoforms of which one is shown to be a1. Recent experiments employing these same protocols and raising specific antibodies from a different population of rats provide evidence for the predominant expression of strychnine-sensitive GlyR isoforms in all time tested. 3H-strychnine binding is higher on spinal cords and cultured spinal cord neurons from E 14 rats while previous experiments on the rat embryonic population showed no detectable 3H-strychnine binding sites on E 14 rat spinal cords or on neurons which existed before four days in culture. Failure to detect glycine by spinal cord neurons from the recent population of rats are also completely blocked by 10 uM strychnine, while this concentration of strychnine blocked responses to glycine by aged neurons from the previous rat population by only 20%.

Polyclonal antibody experiments provide evidence for the expression of a GlyR a2 subunit at early times in culture. These results provide evidence for the expression of the strychninose-sensitive a2 isofrom of the GlyR by embryonic spinal cord neurons from the recent rat population at a time when a different population showed expression of a strychninose-sensitive isofrom of the GlyR. (Supported by NIH and The Robert S. Fink Foundation.)

DIFFERENTIAL REGULATION OF ADRENERGIC RECEPTOR DEVELOPMENT BY SYMPATHETIC IMMUNIZATION. Beth A. Rabinovitz, Neil M. Maier, and Sony C. Landis. Dep. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

Alpha and beta adrenergic receptors (a and b AR) mediate the effects of the sympathetic nervous system in peripheral tissues. Sweat glands are an unusual sympathetic target, since activation of AR during early postnatal development elicits a tonic shift in transmitter propensities in the sympathetic innervation, while the mature innervation elicits sweat secretion through cholinergic stimulation of muscarinic receptors. Sweat glands are an interesting system for the examination of AR expression because postnatal day 21 catecholamines, which alter AR expression in many in vitro models of neurotransmitter receptor regulation, are absent in the sympathetic innervation contains acetylcholine, vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP). Alpha 1B and 1D receptors are present in rat sweat glands, but there is a substantial decrease of 3H-1D receptor binding after P21. This timing raises the possibility that changes in the sympathetic innervation influence receptor expression. Neuronal sympathoectomy causes a partial failure of AR down-regulation, but has no effect on 1B AR expression. Inactivation of the fetal alcohol syndrome, may involve altered function of HO during brain development. Ontogeny of HO activity in the hippocampus (H), frontal cerebral cortex (FC), and cerebellum (C) of the guinea pig at selected prenatal and postnatal ages and the effects of in vitro ethanol exposure were determined. Fetal guinea pigs at gestational day (GD) 51 and GD 63 (term, about GD 68), and adult guinea pigs were used. The microsomal fraction of each brain region was solubilized at 4°C. HO activity by an optimized method which measures the NADPH-dependent formation of CO from 25 μM isocitrate and labeled CO, using a gas chromatographic method to quantitate CO. For each brain region, HO activity (μmol CO/min) in the GD 63 fetus compared with the GD 61 fetus and the adult. There was no difference in HO activity at each developmental age among the three brain regions. In vitro ethanol (25-100 mM) exposure of microsomes did not affect HO activity. The data demonstrate, for the guinea pig, a distinct ontogenetic profile for HO activity in the H, OC, and C. The effect of prenatal and/or postnatal exposure on HO activity in these brain regions is being studied. (Supported by the Medical Research Council of Canada).
T02.13
EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE IN AXOTOMIZED RUBROSPINAL NEURONS
Departments of Anatomy & Neurosurgery, Neurobiology, and Neurochemistry, Harvard Medical School, Boston, MA.

Calcitonin gene-related peptide (CGRP) is a multifunctional neuropeptide expressed in multiple neuronal types throughout the nervous system. CGRP mRNA is expressed in several mesencephalic nuclei of the rat, including the rubrospinal tract. The present study was designed to characterize the expression of CGRP in axotomized rubrospinal neurons. Under conditions of chronic axotomy, the expression of CGRP mRNA was significantly increased in rubrospinal neurons as compared to controls. This increase was observed at both the messenger RNA and protein levels. The increased expression of CGRP mRNA and protein in axotomized rubrospinal neurons suggests a role for this neuropeptide in the regeneration of these neurons. Further studies are needed to elucidate the mechanisms underlying this upregulation and its potential therapeutic implications.

T02.14
DEVELOPMENT OF THE RAT BRAIN HISTAMINE SYSTEM IN RELATION TO THE SEROTONERGIC AND CATECHOLAMINERGIC SYSTEMS
Neuroscience Laboratories, Faculty of Agriculture, University of Tsukuba, Ibaraki 305, Japan.

The development of rat brain histaminergic system was studied using the activity of histidine decarboxylase (HDC), which catalyzes the rate-limiting step in the synthesis of histamine. HDC activity was detected in the developing rat brain at postnatal days 12-15 and was maximal at postnatal day 15. HDC activity was increased in the developing rat brain compared to the adult brain. These results suggest that the development of the histaminergic system is regulated by the activity of HDC and that the histamine system develops in a synchronized manner with other neurotransmitter systems.

T02.15
EXCITATION-SECRETION COUPLING IN CHOLINERGIC NEURONS WITHIN THE EMBRYONIC CHICK HEART. D. B. Gray, G. E. Liebman, L. A. Stahel, G. Pilar, Department of Biology, Simmons College, Boston, MA; Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT, USA.

In the embryonic chick heart, nicotine-induced acetylcholine (ACh) release is mediated by voltage-gated calcium channels (Gray et al., Soc. Neurosci. Abstr., 1994). This release is blocked by alpha7 nicotinic antagonists. In the present study, we investigated the role of calcium channels in ACh release from chick heart ACh release. ACh release was blocked by calcium channel blockers. This suggests that calcium channels are involved in the release of ACh from the embryonic chick heart.

T02.16
DEVELOPMENTAL CHANGES IN THE CHOLINERGIC SYSTEM OF THE RAT BRAIN

The development of the cholinergic system in the rat brain was studied using histochemical methods. The cholinergic system is present in the embryonic brain and develops in a synchronized manner with other neurotransmitter systems. The developmental changes in the cholinergic system were studied using the activity of acetylcholinesterase and the distribution of choline acetyltransferase. These studies suggest that the development of the cholinergic system is regulated by the activity of these enzymes and that the cholinergic system develops in a synchronized manner with other neurotransmitter systems.

T02.17

The development of myelentic neurons in dissociated cell culture was studied using histochemical and immunohistochemical methods. The developmental changes in the myelentic neuron population were studied using the activity of acetylcholinesterase and the distribution of choline acetyltransferase. These studies suggest that the development of the myelentic neuron population is regulated by the activity of these enzymes and that the myelentic neuron population develops in a synchronized manner with other neurotransmitter systems.

T02.18
PRENATAL DEVELOPMENT OF NITRIC OXIDE SYNTHASE IN THE MOUSE SPINAL CORD.
G. Bröning, S. M. M. Meier, B. Mazur.

The prenatal development of nitric oxide synthase in the mouse spinal cord was studied using immunohistochemical methods. The development of nitric oxide synthase was studied using the activity of nitric oxide synthase and the distribution of nitric oxide synthase. These studies suggest that the development of nitric oxide synthase is regulated by the activity of these enzymes and that the nitric oxide synthase system develops in a synchronized manner with other neurotransmitter systems.

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S10A1 is a small, acidic calcium-binding protein, which is expressed in a variety of tissues and cell types, including neurons. S10A1 modulates a number of intracellular processes via interaction with target proteins such as glucogen phosphorylase, aldolase and tau protein. Furthermore, documented increases in S10A1 over-expression have been associated with reduced neuronal survival and cell growth. To determine what cellular processes are regulated by S10A1, stable transfectants of PC12 cells which over- and underexpress S10A1 have been prepared using a mammalian expression vector which contains the S10A1 cDNA in the sense (pSense) or antisense (pAntisense) orientation with respect to a cytomegalovirus promoter.

These DNA constructs were transfected into PC12 cells using electroporation. Reverse transcriptase-polymerase chain reaction analysis demonstrated that three clones expressed the pSense mRNA and three clones expressed the pAntisense mRNA. Indirect immunofluorescence microscopy showed that the expression of the pSense and pAntisense mRNAs resulted in increased and decreased S10A1 levels, respectively, in PC12 cells. Functional studies on these clones will be presented. Furthermore, these studies will provide insights into the consequences of altered S10A1 expression and its role in neurodegenerative processes. This research is supported by a grant from the NIH (30960).

703.2 ACTIVITYPE II IA RECEPTOR mRNA EXPRESSION BY NEURONS OF THE AVIAN CILIARY GANGLION. K. Kos, and J.N. Coyle.* Dep. of Anatomy and Cell Biology, Unv. of Washington, Seattle, WA 98195.

Recent advances in cell culture have suggested that the protein activin may serve as a neuritodifferentiation factor regulating the expression of somatostatin in neurons of the avian ciliary ganglion. We therefore sought to determine if any of the activin receptor isoforms are expressed by developing ciliary ganglion neurons in vivo. Oligonucleotide primers designed to amplify the activin type IA and type IB. Both of these primer sets amplified bands of the anticipated sizes in reverse transcription polymerase chain reactions (RT-PCR) with target RNA isolated from ciliary ganglia. When RNA from a control avian ciliary ganglion was used as the target instead, only primers corresponding to the activin type IA isoform resulted in RT-PCR amplification of an appropriately sized product. This RT-PCR product was sequenced and confirmed its identity as a fragment of the chicken activin type IA isoform. It thus appears that mRNA for the type IA, but not the type IB activin is expressed in the ciliary ganglion.

703.3 EXPRESSION OF FGF ISOMERS IN THE DEVELOPING MURINE NERVOUS SYSTEM.

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Acidic Fibroblast growth factor (FGF-1) and basic FGF (FGF-2) have potent effects on the proliferation and differentiation of neural precursor cells. FGFs mediate biological activity through tyrosine kinase cell surface receptors known as the fibroblast growth factor receptors (FGFRs). A family of four, highly homologous FGF genes (FGFR-1-4) have been identified. Specific sequences encoded by separate exons in the FGF gene are alternatively spliced into the C-terminal half of loop III to create various receptors that have different binding specificities for the FGFs. There is evidence that FGF action is regulated by spatiotemporal differences in the expression of these FGF isomers. We have designed an RT-PCR assay to detect expression of each isomer of the FGFs in the embryonic nervous system and to generate isomer specific cDNA probes for use in RNase protection and in situ hybridization analysis. An RT-PCR assay on mRNA from embryonic day 10 (E10) embryos revealed a total of four FGF isomers produced at E10, i.e. FGFR1, 2, 3, but not of FGFR4. RT-PCR and RNase protection analyses on mRNA from an E10 neuroepithelium derived cell line (call 23D) demonstrated that the expression of FGFR1 gene is very high compared to the expression of other isomers. The FGFIR1 a is the only isomer in this cell line, which is used to postulate the action of FGF-2 (1). The distribution of FGF protein is currently being investigated using antibodies specific to FGFR1, 2 and 3.

1. Brickman, Y. et al., 1995, J.Biol.Chem. (subm)


bFGF, angF, and CNTF lack secretory signal sequences and are primarily cell associated. Trans-synaptic or hormonal stimulation of adenylate cyclase in bovine adrenal medulla (BAMC) leads to nucleotide exchange from GTP to cAMP suggesting a direct nuclear function of bFGF (Stachowiak et al., 1994). To determine how nuclear bFGF could exert its biological effects we examined the subcellular distribution of FGFIR. Immunofluorescence and Western analysis of nuclear biochemical fractions are consistent with bFGF binding to the nucleus, with little cytoplasmic or plasma membrane localization. Nuclear FGFIR is represented by 103 KDa protein containing both the N-terminal (binding) and C-terminal (signaling) domain of the bFGFIR protein. These results are consistent with the hypothesis that bFGF activates the nuclear matrix and nucleolus, but not with chromatin. Electron microscopy revealed patches of FGFIR localized with bFGF patches in the interior of the nucleus. Treatment with forskolin induced parallel accumulation of bFGF, FGFIR and bFGF-TK in the nucleus. Nuclear translocation of bFGF and its receptors offers a novel mechanism for nuclear action (Supported by NIH, NSF, AFOSR).

703.5 characterization of LIF-RECEPTOR (LIF-R) ANTAGONISTS IN BIOLOGICAL ASSAYS. A. B. Vemulapalli, K. Hudson and J. K. Heath.

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LIF-R and gp130 oligomerize and activate intracellular signals in response to LIF, OSM, CNTF/ENT, and CT-L. Given the large number of ligands, antagonists of LIF-R function would be useful in probing the role of LIF-R.

As a first step toward an antagonist, we have isolated a cDNA from a human library that binds ligands, but does not signal, the extracellular domain of LIF-R was truncated after the membrane-proximal hematoxinoprotein domain and fused to the Fc region of human IgG1. This soluble receptor (LIF-R-Fc) was expressed in 293T cells and purified on protein A. In I?/F3 assays the proliferation response to LIF was reduced by 50% at approximately 1 Molar excess of LIF-Fc.

Preliminary results suggest that both these antagonists reduce the LIF-stimulation of the VIP promoter in IMR-32 neuroblastoma cells indicating when they may be useful in studying the function of LIF-R in the nervous system.


Vasoactive intestinal peptide (VIP) has been shown to act through GTP- sensitive binding sites to regulate embryonic growth during the early post-implantation period (Nature, 1993, 362:155, J. Clin. Invest. 1994, 93:2000). A series of VIP analogues that lack VIP's AMP- dependent mechanisms, has recently been shown to enhance neuronal survival (J. Pharm. Exper. Ther. 1995, 273). The purpose of the current study was 1) to examine the action of SNV on the growth of day 9.5 mouse embryo; and 2) to compare the distribution of SNV binding sites in CNS with GTP-VP-sensitive VIP binding sites. SNV and GTP-VP-sensitive binding sites were determined by competition binding assay and localization for SNV and VIP in the presence of 10^-M SNV or 10^-M CMP-PNP (a stable GTP analogue), respectively, on adjacent sections. Treatment of mouse embryos in culture with 10^-M SNV resulted in a significant increase in cell number and cell size. Treatment of mouse embryos grew 3.7 somites during the four hour incubation period compared to 2.2 somites by controls and 5.2 somites by 10^-M VIP. Embryonic growth was not stimulated by treatment with the related peptide PACAP (10^-M to 10^-M). In vitro autoradiography revealed that SNV displaced 125I-VIP only to specific sites in the CNS and these sites coincided with GTP-VP insensitive VIP binding sites identified on adjacent sections. Co-treatment with both GTP and VIP resulted in the total displacement of radiolabeled VIP from brain sections. These data suggest that VIP-induced stimulation of embryonic growth is through a GTP-VP-insensitive mechanism and that SNV acts on GTP-VP-insensitive binding sites in the brain.
ALK-7, A NOVEL BRAIN SPECIFIC SERINE-THERONINE KINASE RECEPTOR. M. Ryden, H. Jernvall, M. Trump and C.P. Dafner. Laboratory of Molecular Neurobiology, MBI, Karolinska Institute, S-171 77 Stockholm, Sweden

Receptors for members of the transforming growth factor beta (TGF-β) superfamily have been classified into three types, from which only type I and II represent signaling receptors with intrinsic serine-threonine kinase activity. To isolate new members of this receptor family specific for the nervous system, PDGF receptors were co-purified with proteins derived from cultured rat P1 brain CDNA. Different fragments showing similarities to both type-I and type-II receptors were found; one corresponds to a previously non-described type-I ser/thr kinase receptor which we have named ALK-7. A full-length cDNA clone was isolated and shown to contain a 494 aa open reading frame displaying all the characteristic functional domains of type I receptors. The amino-acid sequence of ALK-7 shows up to 66% identity to ALK-4 and ALK-5. RNase protection assays and in situ hybridization demonstrated a highly specific pattern of expression restricted to the central nervous system. ALK-7 mRNA was predominantly expressed in postnatal cerebellum and hippocampus. In vivo translation generated a major 55 kDa product, in accordance with the predicted as sequence. Ectopic expression in COS cells and 125I-surface labeling showed ALK-7 to be a surface protein. Receptor reconstitution and cross-linking experiments are currently being performed to determine whether any of the known members of the TGF-β ligand superfamily, including TGF-β1, TGF-β2, activin, bone morphogenic proteins (BMPs) and gial cell line-derived neurotrophic factor (GDNF), is a ligand for ALK-7.

ANAALYSIS OF MUTANT PDGF RECEPTORS EXPRESSED IN PC12 CELLS IDENTIFIES SIGNALS GOVERNING NA CHANNEL INDUCTION DURING NEURONAL DIFFERENTIATION. G.R. Grad, K. L. Heasley I, J.P. R. Losa, A.C. E. Iwamasa, H., L.P. M. Moroney, D. L.P. J. Johnson, 4 and R.A. Map* I, Departments of 1Biochemistry and Physiology, Dartmouth Medical School, Hanover, NH 03755, 2Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, and 3Department of Pharmacology, Univ. of Colorado Medical School, Denver, CO 80262

We have previously shown that stimulating tyrosine kinase receptors play an important role in neuronal differentiation, the mechanisms by which this occurs are not well understood. To identify the signals necessary for the induction of voltage-dependent Na channel expression, a crucial aspect of neuronal differentiation that can occur independent of p21ras activation, patch clamp analysis and RNase protection assays were used to assess effects of expression plasmid-derived growth factor (PDGF) receptors with mutations in tyrosine residues of the cytoplasmic domain that associate with various signaling molecules upon activation of the receptor. Mutations that block activation of PD3-K, PLCγ, GAP, and Syr did not alter the PDGF-mediated induction of Na channel expression. However, mutation of tyrosines that associate with members of the src family of kinases significantly inhibited induction of functional Na channel expression in response to PDGF. Furthermore, mutations that block association with members of the src family of kinases were found to be necessary for the induction of mutations that block PD3-K, PLCγ, GAP, and Syr activation, abrogated the PDGF-mediated induction of type II Na channel a subunit mRNA, indicating that multiple signals generated by the receptor contribute to this response. The results were due to the specific mutations introduced and not simply the result of an overall decrease in receptor kinase activity, since transf and c-fos mRNA were still induced by all of the mutant receptors analyzed. The results provide insight into the signal transduction mechanisms governing expression of type II Na channel a subunit mRNA and Na current density, and mechanisms underlying neuronal differentiation. Supported by NS36767 to RAM.

GANGLIOSESIDES REGULATE CYTOSKELETAL STRUCTURE AND PROTEINS IN NE URO-2a NEUROBLASTOMA. L.J. Wang, R. Codella & F.J. Rossen A. Anatomical Sciences & Neuroweb, Univ. of Louisville School of Med., Louisville, KY 40202.

It is well established that gangliosides enhance neurogenesis of primary neuronal tissues and several neuronal cell lines, the exact mechanism remains unknown. Our previous studies demonstrate that ganglioside exposure simultaneously increases the microtubule network and neurogenesis of Neuro-2a cells. A redistribution of MAP2 immunoactivity from the perikaryon to the distal processes occurs following 24 hr GM1 treatment. In contrast, MAP5 and tau immunolocalization remains unchanged. Immunoelectron microscopy studies confirm that MAP2 is more closely associated with actin-rich subcortical cytoplasm than with microtubules after GM1 exposure whereas actin becomes dispersed from distinct regions to the neuron, protein and RNA analysis is in progress. Western blot analysis reveals increased levels of acetylated tubulin but not MAP2 following GM1 exposure. Actin increases in a GM1 dose dependent fashion. Studies in progress examine the time dependent effects of GM1 exposure on actin levels and topography via immunolocalization. Our data suggest that gangliosides may be involved in the determination of dendritic or axonal fate by selectively changing the distribution of MAP2, stabilizing microtubule networks and increasing actin synthesis. It is possible that gangliosides enhance neurite formation by altering the balance of microtubules and microfilaments through MAP2-dependent mechanisms. Supported by Alliant Community Trust Fund, Louisville, KY.

PREGNENOLONE SULFATE INCREASES THE GROWTH OF HIPPOCAMPAL NEURONS. R. D. Brown* and R. Gunawan, Dept. Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles, CA 90033.

Pregnenolone sulfate (PS) has been found to be synthesized de novo in oligodendrocytes of the hippocampus and cortex thereby providing locally synthesized concentrations of this neurosteroid. PS has also been found to enhance memory functions in mice and rats. Growth in the projections and synaptic connections has been postulated to be one of the fundamental cellular mechanisms involved in learning and memory. Although experiments have clarified the impact of PS on the growth and morphology of hippocampal nerve cells. Results of this investigation demonstrated that PS induced a significant increase in the number of neurites and the total length of neurites (p<.05, ***p<.01), in the total branch length (p<.05, ***p<.001), in the number of bifurcation points emanating from neurites (p<.05, ***p<.001) and in the number of microspikes (p<.05, **p<.01, ***p<.001). We pursued the mechanism of PS-induced nerve cell growth by investigating the impact of blocking the NMDA glutamate receptor on PS-induced hippocampal nerve cell growth. Preliminary results of these experiments indicate that the growth promoting effects of PS (500 nM. **p<.01) are completely abolished in the presence of the NMDA receptor antagonist AP5 (10 μM). In addition, our results indicate that NMDA receptor activation is not required for normal nerve cell growth, since the growth of neurites in the presence of AP5 was not significantly different from control, but is involved in the potentiation of normal nerve growth. Results of these investigations indicate that the neurosteroid, PS, can act as a significant neurotrophic factor for hippocampal neurons. Supported by grants from the National Foundation and the Schuster Foundation to R.D.B.

ANALYSIS OF RECEPTOR TYPE TYROSINE KINASE AND ITS LIGAND DURING EARLY MOTONEURON DEVELOPMENT. K. Ohta*; N. Ikeda; S. Nonaka, H. Taniuchi, R. Suzuki, and N. Oka{s}a, Dept. of Neurobiology and Immunology, Kuma University Grad. Sch. of Med. Sci., Kumesato 862, Japan.

Receptor tyrosine kinases (RTKs) play important roles in cellular survival, proliferation, and differentiation. We have isolated 19 RTK receptors by RT-PCR method from E14 chick motoneurons. In situ hybridisation analysis revealed that chick Sk is a member of eph family, was expressed from E5 on motoneurons at the brachial and lumbar segments of the spinal cord which innervate limb muscles, that disappeared after the naturally occurring cell death period (E9-E11). Immunohistochemistry using anti-chick Sk monoclonal antibody showed the localization of chick Sk protein at cell bodies and axonal fibers of motoneurons. We also isolated a chick Sk like ELF-I receptor, which was identified as a ligand for both Mek4 and mouse Sk. Chick ELF-I was 72% identical to mouse ELF-I. While the expression of chick Sk was highly regulated in time and space, that of ELF-I was widespread. The unique expression of chick Sk suggests the involvement of it in the cell-cell interactions for specific subpopulations of developing motoneurons.

Phosphatidylinositol (P1-3) kinase is implicated in the regulation of diverse cellular processes including neurite outgrowth in PC12 pheochromocytoma cells. P1-3 kinase is composed of a 110-kDa catalytic and an 85-kDa regulatory subunit. Here we describe p56(k), a new regulatory subunit that was isolated by screening expression libraries with tyrosine phosphorylated IRS-1. The p56(k) is composed of a unique 30-residue NH2-terminus followed by a proline-rich motif and two SH2 domains which contain homology to those of p56.

In contrast to the ubiquitous expression of the previously described p56, expression of p56(k) is mainly in the central nervous system (CNS). Maximum levels of expression are found between 13.5-15.5dpce, decreasing in subsequent developmental stages with only minimal levels detected in the adult brain. High levels of p56(k) expression are temporally and spatially coincident with neuronal differentiation in CNS development.

The p56(k) forms a stable complex with p110, and associates with IRS-1 during processing in both primary neuronal cultures and in P19 cells differentiated to neuronal fate. The unique features of p56(k) suggest that it may be important in IGF-I receptor signaling during neuronal differentiation.


Hepatocyte growth factor (HGF)/scatter factor (SF) is a multifunctional cytokine that stimulates mitogenesis, motogenesics, morphogenesis of a broad spectrum of epithelial and endothelial cells. The HGF/SF cellular responses are mediated by four transmembrane kinases. Three of these kinases display a similar biological function(s), each having the HGF/SF and its receptor in the nervous system, we chose to analyse the expression of the HGF/SF and its receptor in primary culture of sympathetic neurons from the superior cervical ganglia (SCG). RT-PCR demonstrated that sympathetic neurons express HGF/SF mRNA. Moreover, condition media of sympathetic neurone cultures contain bioactive HGF/SF which is needed for scatter activity and MDCK cells. Sympathetic neurones also synthesise met receptor mRNA, and immunofluorescence studies indicate that the Met receptor is localized to both cell bodies and neuritic processes. Thus, sympathetic neurones synthesize both HGF/SF and its receptor, Met, suggesting the possibility of a novel autocrine loop. The possible biological function of such a circuit may be under investigation in sympathetic neurones in culture and in vivo.


Intracellular domains of receptors can act as autonomous signalling units. As a means of assessing p75 neurotrophin receptor signalling functions in vivo, we have introduced a series of truncated p75 neurotrophin receptor (p75(T1)) in transgenic mice. We have produced lines which overexpress the intracellular domain of p75 (ICD) within neurons. Specifically, we have utilised the Tau-tubulin promoter, which is expressed at high levels in early developing neurones, and shows reduced expression after neurons have differentiated. Analysis of four lines of transgenic mice, using the Tau-tubulin construct revealed that at least two of these lines express the ICD at readily detectable levels. Animals from both lines display pros and cons that are smaller and noticeably less coordinated than their control littermate. Analysis of these lines, the sensory and sympathetic ganglia is significantly smaller than in control littermates. Cell counts revealed that transgenic sympathetic ganglia contain approximately 50% fewer neurons than control littermates. Analysis of sensory neurones of the DRG indicated 60% cell loss, preferentially of small neurones. To determine whether the CNS of these animals is also affected, Tau-Tubulin ICD mice were crossed with a previously characterized line of transgenic mice that expressed flg-galactosidase from the same promoter (line K6, TutLaAzC, animals as reported in Glotzer et al. 1.

Neurol., 14, 7319, 1994). Histochemical staining with Xgal revealed that ,...are significant perturbations in neuronal distribution throughout the brains of the Tau-Tubulin ICD mice. For example, the laminar distribution of neurones within the cortex is disturbed and occasional clusters of blue neurones are observed within the white matter. The cellular basis for these perturbations in neuronal development is currently under investigation.


We have previously reported that interleukin-3 (IL-3) is one of the candidates for cholinergic neurotrophic factors (Neuron 4:429-436, 1990). Since that report, it has been questioned whether IL-3 acts directly on cholinergic neurites or indirectly on other populations of cells in the central nervous system (CNS). We now demonstrate the expression of IL-3 receptor (IL-3R) alpha (SP-1) and beta (IL-3R) in the CNS neurons by means of reverse transcription and polymerase chain reaction (RT-PCR) and immunohistochemistry, although the partial data on IL-3R beta were already reported (Neurosci. Res. 19:3132, 1994; Int. J. Dev. Neurosci. 19, in press). RT-PCR followed by Southern blot analyses revealed that the mRNA for IL-3R alpha and beta are found in an embryonic cholinergic septal cell line SN6G and in postnatal neurones derived from the septal regions of embryonic mice. In contrast, a postnatal cholinergic septal cell line SN52 expresses none of the mRNA for IL-3R alpha and beta (SN6G, SN52 are kind gifts from Dr. B. Wanner, Albert Einstein College of Medicine, Bronx, NY). Actually IL-3 elevates choline acetyltransferase (ChAT) activity in SN6G but not in SN52. These results indicate that IL-3 is utilized by cholinergic neurones via a set of IL-3R alpha and beta expressed in the cholinergic neurones. The results were supported by immunohistochemical study showing that IL-3R alpha and beta were found in cholinergic neurones of the basal forebrain. (Supported partially by the Funds from the Science and Technology Agency, the Health Sciences Foundation, and the Naito Foundation in Japan.)

Cytoplasmic Homology Between p75LNT and TNF-R1 and the Fas Antigen May Define Analogous Functional Domains. P.A. Barker, C. Zeidler, D. Miller, and T. Ireland.

A novel cytoplasmic domain (C-terminal) of TNF receptor 1 (TNF-R1) and the Fas antigen may define analogous functional domains. This conclusion is based on the following observations: 1) the cytoplasmic domain of each receptor is immunologically conserved in evolution; 2) the cytoplasmic domains of both receptors are capable of self-association, interaction, and may be involved in the regulation of nuclear transcription factors; 3) there is an extensive homology in amino acid sequence; and 4) the functional domains of both receptors are conserved between the TNF-R1 and Fas.


Developmentally-regulated expression of the Leukocyte Common Antigen-Related (LAR) tyrosine phosphatase receptor and neuron-preferential alternative splicing suggests that LAR regulates mammalian neural development (Longo et al. 1995). LAR signalling involves a complex array of brain RNA from LAR homologous (L-) transgenic mice showed trace or absent ~8kb LAR transcript in comparison to the wild type (~11kb, DBA2). Dendrite cytoarchitecture from basal forebrain was examined by immunohistochemistry using antibody against the p75 NGF receptor which localizes with cholinergic markers. In p75(-/-) mice we observed: 1) reduction of p75 immunoreactivity in the infragranular band; 2) disrupted laminar pattern of fibers entering the granule cell layer; and 3) fiber thickening with aberrant budding in granular and polymorphous layers, raising the possibility of a neurodegenerative and prune/culturatrophy neuropathies. These experiments intercrosses will determine if strain difference account for any of these abnormalities. These observations support the hypothesis that LAR and p75 act to maintain normal dendrite development and may also provide insight into the mechanisms by which LAR and p75 influence neuronal differentiation.
LAR TYROSINE PHOSPHATASE RECEPTOR: RNA AND PROTEIN EXPRESSION OF LAR ALTERNATIVELY SPliced ELEMENT-C (LASE-C).}

The Leukocyte Common Antigen-Related (LAR) tyrosine phosphatase receptor contains Ig and fibronectin type III domains resembling those in cell adhesion molecules mediating neurite outgrowth. Developmentally regulated expression of rat LAR in neurons suggests a role in neural development (Longo et al., 1993; Longo et al. BJC, 1993). LASE-C is a 27 bp alternatively spliced exon in the fifth fibronectin type III LAR domain. Its splicing is regulated by development, NGF and demeretation and preferentially occurs in neural tissue (Zhang and Longe, 1995). In-situ hybridization with a LASE-c probe which identifies the ~7 kb LAR transcript on Northern blots showed diffuse neuronal expression at birth. In adults, however, expression was largely restricted to subsets of neurons in the hippocampus and vestibular, reticular thalamic, ocoulomotor and deep cerebellar nuclei. Northern analysis showed markedly reduced LASE-C expression in cortex, hippocampus and cerebellum during postnatal development. On Western blots, affinity-purified antisera raised against LASE-C peptide identified the predicted 150KD LAR band and preferentially stained neurons in dorsal root ganglia. LASE-C antibody also stained scatic nerve neurites and neurites and growth cones of cultured neurons. Expression of LASE-C in specific subsets of neurones and presence of LAR protein with the LASE-C domain in neurites and growth cones supports our hypothesis that highly-regulated alternative splicing of LAR-type tyrosine phosphatases regulates neurite outgrowth and/or synapse formation. Supported by American Paralysis Association (FL) and NIA R01 (FL).

INTERLEUKIN-1ß RECEPTOR MEDIATED NGF SECRETION FROM RAT CORTICAL ASTROCYTES IN PRIMARY CULTURES.}

In interleukin-1ß (IL-1ß) is one of the most potent stimulators of NGF secretion from rat neonatal cortical astrocytes in primary cultures. Our previous studies showed that IL-1ß acts at the level of NGF gene transcription and suggested giall IL-1 receptor mediated mechanism (Carman-Krzan and Wise BC. J Neurosci Res. 34, 1993, 225-232). Using sodetated recombinant human IL-1ß in the binding studies we identified IL-1ß receptors on the astrocyte cell population (Kd=2.6 x 10^-9 M, nM). Further characterization of IL-1ß receptors on astrocytes with inhibition binding studies showed that rh IL-1ß is the most potent inhibitor of NGF secretion followed by rh IL-1ßa (Kd=2.0 x 10^-9 M, nM). rh IL-1a (Kd=7.9 x 10^-9 M, nM) and its IL-2 alone failed to stimulate NGF release. Our further studies showed that rh IL-1ß stimulated NGF release is inhibited by the presence of rh IL-1ßa in a dose dependent manner, confirming that IL-1ß induced NGF secretion from astrocytes is IL-1ß receptor mediated process.

Role of Interleukin 1ß converting enzyme in neuronal cell death.}

Valeria Gagliardini, Weiwei Li, Mark Fishman, Yunjun Yuan Cardiovacular Research Center, Massachusetts General Hospital,149 13th Street, Charlestown, MA 02129

Interleukin-1ß converting enzyme (ICE), the mammalian homolog of ced-3, is formerly known as the enzyme responsible for the production of IL-1ß. Recently our studies demonstrated that ICE activity participates in neuronal cell death (CD) and inhibition by crmA can delay this process (Gagliardini et al., 1994). In order to better understand the role of ICE in programmed CD, we demonstrated an induction of ICE during apoptosis in dorsal root ganglion (DRG) neurons. A basic level of ICE was detected in neurons and the expression of ICE was upregulated in dying neurons with condensed nuclei. The requirement of ICE-ced-3 family genes in neuronal programmed CD is supported by the ability of the precursor of the IL-1ß (pIL-1ß) or mutant ICE with a point mutation in ICE active site IC to G), that eliminate pIL-1ß processing activity as well as autocaprocessing, to prolong neuronal survival. Microinjection of the human pIL-1ß gene as well as the equivalent of mouse ICE into chicken DRG neurons was found to prevent CD induced by deprivation of nerve growth factor. Neurons were able to survive for 6 days off organs with neurites and healthy cell body. These finding suggest a role for ICE playing in neuronal cell death. Further experiments have to be done to understand whether additional members of the ICE-ced-3 family are also involved in controlling neuronal CD.

GENE EXPRESSION IN NEURONS DURING PROGRAMMED CELL DEATH.}

R. S. Freeman and J. C. Crowder. Department of Pharmacology, University of Rochester, School of Medicine, Rochester, NY 14642.

The expression of certain genes is upregulated during programmed cell death (PCD) in sympathetic neurones deprived of nerve growth factor (NGF) (Freeman et al.) Neuron 12:343-355, 1994; Eliaus et al., J. Cell. Biol. 127:1717-1727, 1994). These genes are induced despite an overall decrease in RNA and protein synthesis soon after NGF withdrawal. To identify other genes that are preferentially expressed in dying neurones, we have utilized the technique called differential display as a means of comparing the mRNA present in NGF-maintained versus NGF-deprived neurones. With this technique, we have identified a new gene, termed DD-1(X), that is upregulated after NGF withdrawal. DD-1(X) mRNA levels begin to accumulate within 3 hours after NGF withdrawal and peak at about 10 hours. To test the validity of DD-1(X) and other genes, such as cyclin D, c-jun, c-fos, and c-myc in the cell death process, we have examined their expression in neuronal death paradigms that are independent of NGF withdrawal. For example, cyclic ammine (Ac) kills postmitotic neurons in a manner that resembles NGF deprivation except that the onset of death is delayed by many hours. DD-1(X), cyclin D1, c-myc, c-fos, and c-jun are all upregulated during Ac-induced neuronal death. Furthermore, the induction of these genes is delayed relative to their induction during NGF deprivation-induced PCD, consistent with their potential involvement in the cell death process. To test DD-1(X) and cyclin D1 for a direct role in the mechanism for PCD, we are attempting to inhibit their expression in NGF-deprived neurones using antisense approaches.
T04.3

PC12 CELLS, TRANSPLANTED WITH CALBINDIN-D28K CDNA, ARE PROTECTED FROM DEGENERATION CAUSED BY SERUM WITHDRAWAL. A. McMahan, E. Lephalt*, C. Li, Liang, and D.C. Germain, Dept Psychiat, UT Southwestern Med Sch, Dallas, TX 75235-9070.

T04.4


We have used both the PC12 cell line and primary cultures of rat sympathetic neurons as model systems to examine the mechanism by which neuronal cells undergo apoptosis. Death in these neurons follows an uncoordinated cell cycle progression and apoptotic death similar to that found in other cell types. We have developed a system to test these neurons for their ability to protect PC12 cells and sympathetic neurons following trophic support withdrawal. Two such agents S-nitroso-N-acetylcysteine (SNAP) and dinitrophenol (DNP) provide complete protection for at least two days following removal of serum from naive PC12 cells while only 15% of the untreated cells remained alive. These compounds also provide complete protection for two days to neuromodulator PC12 cells following withdrawal of SNAP in serum-free medium while only 50% of the untreated cells survived. Treatment of primary sympathetic neurons with DETA-NQ and SNAP following withdrawal of NGF also resulted in significantly enhanced survival relative to controls. A well understood target of NO action is guanylate cyclase which is activated by nitrosylation of its heme prosthetic group. LY-83,583, an inhibitor of guanylate cyclase, blocks the protective effects of both SNAP and DETA-NQ on serum-deprived PC12 cells without preventing NGF-induced survival, suggesting that the protective effects of NO may be mediated by elevated intracellular cGMP (Supported by grants from the NINDS).

T04.5

INDUCTION OF p33-DEPENDENT NEURONAL APOPTOSIS BY THE ANTIMITOTIC CYTOSINE ARABINOSIDE, WHOSE TOXICITY CLOSELY RESEMBLES DEATH BY TROPHIC FACTOR DEPRIVATION. T.J. Deckweth* and F.M. Johnson, Jr., Washington University School of Medicine, Department of Molecular Biology and Pharmacology, St. Louis, Missouri 63110, USA.

Exposure of nerve growth factor (NGF)-maintained neonatal rat sympathetic neurons to the antimitotic cytosine arabinoside (araC) resembles death by NGF deprivation based on pharmacological criteria (Martin et al., J. Neurosci. 10: 184-193, 1990). Here we show that the similarities between death by NGF deprivation, which occurs by apoptosis (Deckweth and Johnson, J. Cell Biol. 123: 1207-1222, 1993) and death by exposure to araC extended to several biochemical and morphological parameters indicative of apoptotic neuronal death. Similar to NGF deprivation, araC-induced apoptosis was accelerated in murine sympathetic neurons deficient in the death suppressor bcl-2. To determine whether araC might induce apoptosis by inflicting DNA damage, we examined whether the gene dose of the tumor suppressor p53, which triggers apoptosis upon extensive DNA damage, would affect the kinetics of death after treatment with araC. In the absence of p53, neuronal death by exposure to araC was delayed significantly, consistent with a potential role for DNA damage in contributing to araC-induced neuronal apoptosis. Supported by the AT Children's Project and NIH grant NS24769.

T04.6


We have previously reported that transcription factor AP-1 DNA binding activity transiently increased and then decreased following apoptotic cell death induced by serum deprivation. NGF, which rescues PC12 cells from apoptosis, upregulated AP-1 binding activity persistently. Our hypothesis is that impaired AP-1 DNA binding activity after serum deprivation may contribute to apoptosis in PC12 cells and that NGF may rescue cells via maintenance of AP-1 DNA binding activity. K252a, an inhibitor of the Trk receptor, abolished NGF rescue of PC12 cells and increased AP-1 DNA binding activity. Enhanced AP-1 activity in the presence of both NGF and protein synthesis inhibitor cycloheximide treatment indicated that there is persistent AP-1 activity activation due to posttranslational modifications of various fos and jun proteins. Increased AP-1 DNA binding activity also was persistent during insulin and cAMP-mediated survival of PC12 cells after serum deprivation. In addition, there was a decline in AP-1 activity in PC12 cells after NGF deprivation. Furthermore, retinoic acid, known to have a repressive function on AP-1 activity, abolished NGF-mediated rescue and increased the sensitivity of PC12 cells to serum deprivation. Therefore, serum deprivation-induced cell death and NGF rescue correlate with AP-1 DNA binding activity. Supported by NINDS NS18708. This is publication 35A from USPHS grant PO1 AG10514 awarded by NIA.

T04.7


We have used serum-deprived PC12 cells and NGF-deprived sympathetic neuron cultures as models to study apoptosis elicited by trophic factor withdrawal. In our paradigm, N-acetyl-cysteine (NAC) maintains in vivo cell survival of PC12 cells and sympathetic neurons and also inhibits PC12 cell proliferation. NAC has been reported to be an anti-oxidant that increases intracellular glutathione (GSH) levels. However, several other anti-oxidants do not promote survival or block proliferation, suggesting that NAC's actions cannot be explained by its anti-oxidant properties. The NAC-mediated increase in intracellular GSH also seemed unnecessary, since increasing intracellular GSH with buthionine sulfoximine did not affect NAC's protective or anti-proliferative effects. An alternative explanation is that NAC acts as a reducing agent. When we tested other compounds with reducing properties, a number retarded death. Among the most effective was 2,3 diaminopropanoic acid (British anti-Lewisite). This compound also blocked PC12 cell proliferation. These findings suggest that NAC prevents death by means of its reducing activity and that other reducing agents may also inhibit the apoptotic pathway. Finally, alpha lipoic acid, a naturally occurring antioxidant that blocks DNA synthesis and this activity may account for their capacity to rescue neuronal cells from apoptotic death. Supported by the NINDS and ALS Foundation.

T04.8

CHANGE OF INTRACELLULAR CALM LEVELS IN SYMPATHETIC NEURONS UNDERGOING PROGRAMMED CELL DEATH. L.J. Chang and Y. Kato, Dept. Anayst, Univ. for Arkansas Medical Sciences, Little Rock, AR 72205.

The survival and proper functioning of sympathetic neurons is dependent on nerve growth factor (NGF). When immature sympathetic neurons are deprived of NGF, they undergo an "active" dying process usually termed "programmed cell death" (PCD) or "apoptosis". Cyclic AMP and cAMP-dependent protein kinase (PKA) of intracellular cAMP can prevent this sympathetic neuronal PCD caused by trophic factor deprivation. This trophic dependence is age-related such that the cells become less dependent on NGF as they mature. Sympathetic neuronal cultures derived from rat superior cervical ganglia were used to study the relationship between intracellular cAMP levels and neuronal cell death. With a cAMP antagonist, drugs were given to separate the effects of cAMP without cAMPidal agents and blocked cAMP or its second messenger in mature neurons. These data indicate that as neurons matures, the relative amount of cAMP per cell increases concurrently. There is a 2-fold increase of cAMP levels per cell when the cAMP levels from a 1-week- and 2-week-old culture were compared. Removal of NGF in 1-week-old cultures, which triggers the process of PCD, results in a decrease of intracellular cAMP levels. NGF deprivation for 24 and 48 hours leads to a 50% and 75% decrease of cAMP levels. In contrast, when these cells mature in culture and become relatively NGF independent (i.e., 2-week-old culture), NGF withdrawal does not lead to a drop of cAMP levels. PACAP (pituitary adenylate cyclase-activating peptide) - a naturally occurring bioactive peptide, can increase cAMP levels in these sympathetic neurons, and prevent neuronal cell death resulting from NGF deprivation. (Supported by NIH NS32283)

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METABOLIC AND GENETIC ANALYSES OF APOPTOSIS IN POTASSIUM-DEPRIVED CEREBELLAR GRANULE CELLS T. M. Miller* and M. Johnson. Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110. Cerebellar granule cells may be maintained in serum-containing medium by elevating (25 mM) extracellular potassium levels. Removal of both potassium and serum after seven days in culture triggers a characteristic apoptotic death (D’Mello et al., 1993, P.N.A.S. 90, 10989–10993) We have found that removal of the serum has an important function in this model of apoptosis. After 96 hours of potassium deprivation in the presence of serum, 40–45% of the cells are still alive whereas more than 55% of the potassium/serum-deprived cells have died by this time. Thus, while the newly complete cell death of the potassium/serum-deprivation system provides a convenient model, this system is complicated by removing two sources of trophic support at the same time.

Metabolic and genetic parameters are being analyzed in the potassium/serum-deprivation model. Protein synthesis decreases precipitously after potassium/serum-deprivation to less than 15% of control (25mM K+ plus serum) at 12 hours, a time when approximately 50% of the neuronal cell bodies are committed to die. Cells maintained in 25mM K+ without serum showed a slow decline in protein synthesis to approximately 50% at 24 hours. C-Jun, a gene required for cell death after trophic factor deprivation is also induced several fold at potassium/serum-deprived cerebellar granule cells. Similarities and differences in the pattern of gene expression in dying sympathetic and granule neurons will be presented. Supported by NIH grants NS 24679 and AG 12947 and by the Washington University ADRC (P50 AG 5681).

SEVERE SUBSTITUTE FOR ENDGENOUS FACTORS INVOLVED IN CELL DENSITY-DEPENDENT SURVIVAL OF RAT CORTICAL NEURONS. Y. Yasaki,1,2 L. Ueda,1 H. Fukushira,1,3 T. Okada1,2,3 1Department of Pharmacology, Yokohama City University School of Medicine, Yokohama, 236, Japan, 2International Research Laboratories, Ciba-Geigy Japan, Takarazuka 665, Japan. We have reported that survival of neurons was cell density-dependent in the primary culture of rat cortex under serum-free condition in this meeting last year. To examine mechanism of neuronal cell death at low density culture (1 x 10^3 cells/cm²), we developed a new colorimetric assay for survival, using Alamar Blue. Alamar Blue is a redox indicator which yields a color change in response to chemical reduction derived from living cells. At low density culture, the rate of neuronal survival decreased to 20% of initial cells at 15 hr. In this process of neuronal cell death, genomic DNA laddering was observed, indicating that neurons are dying in a manner of apoptosis. On the other hand, more than 80% of initial neurons survived at higher density culture (5 x 10^3 cells/cm²) at 15 hr. In the presence of 10% serum, however, survival of neurons increased as low density cultures were unchanged at high density. As a result, there was no more density-dependent survival. Survival of neurons at low density was increased by addition of 10% serum only for initial 1 hr and deprivation of serum subsequently, while the survival was not enhanced by the addition of serum at 1 hr after seeding. These findings suggest that serum substitutes for endogenous factors involved in cell density-dependent survival of neurons.

CEP1347 PREVENTS CELL DEATH INDUCED BY NGF-DEPRIVATION IN DORSAL ROOT GANGLION NEURONS AND PC12 CELLS IN CULTURE M. A. Glischna1,*, M. E. Forbes2, T. O’Keefe3, C. Murakata4, N. T. Nen5, B. B. Bucy6, G. C. Strohmann7, K. W. Mumm1, K. D. Buzsko1, C. J naprawa8. 1Department of Biology, West Chester Univ., PA 19380; 2Kyowa-Hakko Kogyo, Tokyo, Japan. Dorsal root ganglion neurons and PC12 cells maintained in the presence of nerve growth factor (NGF), undergo cell death upon its removal. The indolcarboxaldehyde, K-252a, and a structurally novel analog, CEP1347, prevents cell death and associated necroptosis in a variety of neurons in vitro (GL Neuronal. 61:201.1993.S.1502.10). In addition, CEP1347 prevents embryonic and postnatal programmed motoneuron death in vivo and prevents the decrease in DAT immunoreactivity in adultized axonal hypoglycosylated motoneurons (Soc. Neurosci. Abstr. 1994. 1995). To determine effects of such small “neurotrophic” molecules on cell death thought to be primarily due to NGF deprivation, the two systems were employed. In chick embryonic (E9) dorsal root ganglion cultures, K-252a and CEP1347 rescued >50% of the neurons lost after NGF withdrawal (compared to rescue by readdition of NGF). K-252a, a related compound that has no direct effect on the survival and/or differentiation of neurons in vitro, showed no protective effect. In PC12 cultured cells from death after NGF withdrawal, while KT5823, a non-neurotrophic analog, had no effect. Acinxomin D partially reduced cell death in both models, suggesting that the drug’s effects are due to regulation of RNA synthesis, and a CAMP analog (8-(4- chlorophenylthio)CAMP) prevented cell death in both models. These data support previous findings that CEP1347 prevented NGF deprivation induced cell death, and these models is thought to be primarily due to apoptotic mechanisms, CEP1347 may interfere with this process.

BASIC FIBROBLAST GROWTH FACTOR PREVENTS THE LOW POTASSIUM-INDUCED APOPTOSIS IN THE CULTURED CEREBELLAR GRANULE CELLS. H. Saito1,2 and N. Nishiyama1,2. 1Dep. of Chem. Pharmacol., Fac. of Pharmaceutic Sci., The Univ. of Tokyo, Tokyo 113, Japan. Apoptosis, which is characterized morphologically by chromatin condensation, and cytoplasmic and nuclear shrinkage, is one of the pathways of cell death. Cultured mature cerebellar granule cells, isolated from 8-day-old postnatal rat, die by apoptosis when deprived of high extracellular concentration of potassium (25 mM). We have investigated the effect of basic fibroblast growth factor (bFGF) on this neuronal apoptosis. Exposure of cerebellar granule cells to bFGF (0.01-10 ng/ml) protects cultured cerebellar granule cells (DIV 7), maintained in the presence of physiological concentration of potassium (5 mM), from apoptotic cell death in a concentration dependent manner. This antiapoptotic effect was not mimicked by several other nongrowth factors, which are growth factor and brain-derived neurotrophic factor at the same range of concentration. The trophic action of bFGF was remarkably and significantly blocked by tyrosine kinase inhibitors, herbimycin A and lavendustin A. Protein kinase C inhibitors, staurosporine and H-7, also reversed the protective effect of bFGF, whereas protein kinase A inhibitor, H-89 had no effect. Moreover, neither ionotropic nor metabotropic glutamate receptor antagonists block the antiapoptotic effect of bFGF. These results suggest that bFGF prevents the neuronal apoptosis in cultured cerebellar granule cells through activation of FGFR tyrosine kinase and protein kinase C. It is interesting to speculate that FGFR or FGFR-like molecules are released in para- or autocrine fashion to prevent the apoptotic cell loss of granule cells in a living rat cerebellum.
0704.15
PROTEIN KINASE INHIBITION INDUCES APOPTOSIS IN DEVELOPING RETINAL NEURONS. L. Colombaioni * Istituto di Neurofisiologia del CNR, 56100 Pisa, ITALY
Isolated retinas from newborn Ps-Pc rats are an useful model system for the study of trophic-dependent selection process that occurs during early phases of neuronal development. Culture conditions have been found in which, following serum withdrawal, retinal ganglion cells rapidly undergo death showing morphological and functional characteristics of apoptosis. It is known that neurotrophins promote survival of developing neurons by binding to Trk receptor protein tyrosine kinase, but it is also known that the direct activation of protein kinases often fails to inhibit apoptosis. At the aim of further investigate the role of protein kinase pathway in neuronal death, retinas isolated in serum free medium have been exposed for 3-6 hours to protein kinase inhibitor staurosporine 50-100 nM, a dose considered to inhibit selectively protein kinases C. This treatment remarkably accelerated the time course of ganglion cell death. Under these conditions of protein kinase C inhibition, survival promoting action of serum was lost. These data indicate that prevention of neuronal apoptosis requires activation of protein kinase C pathway. Since other reports indicate that this is not sufficient to prevent neuronal death, it is suggested that simultaneous activation of multiple signaling pathways could be required for neuronal survival.

0704.17
PHARMACOLOGICAL AGENTS THAT MODIFY OXIDATIVE STRESS PROMOTE MOTONEURON SURVIVAL DURING DEVELOPMENT. R.W. Oppenheim * J. Hong, J. Caldeiro, C. Milligan, A. Newhouse, L.Lloyd and D. Prevette. Wake Forest University Medical School, Winston Salem, NC 27157
Developing spinal motoneurons (MNS) in the chick embryo undergo normal programmed cell death (PCD) in vivo and following trophic factor deprivation in vitro. Previous in vitro studies of other neuronal and non-neuronal cells have suggested that increased oxidative stress may be involved in the pathway leading to PCD. We have begun to examine whether oxidative stress is also involved in the normal PCD of chick MNS in vivo and in vitro. Preliminary experiments with one anti-oxidative agent, N-acetylcysteine (NAC), indicates that treatment of chick embryos in ovo during the normal period of MN cell death significantly reduces the number of dying (pyknotic) cells and increases the number of healthy MNS present at the end of the cell death period. Studies are in progress to examine other anti-oxidants and to determine their mode of action (and that of NAC) in preventing the PCD of MNS.

0704.18
MECHANISMS OF THE OSMOProtective EFFECT OF IGF-I IN HUMAN NEUROBLASTOMA CELLS. C.C. Matthews* and E.L. Feldman. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109-0888
We have previously shown that exposure to media made hyperosmotic by the addition of mannitol or NAC significantly decreased the number of viable SH-SY5Y human neuroblastoma cells within 24 hrs. In contrast, low concentrations of IGF-I preserved cell viability under hyperosmotic conditions. In the current study, we have examined the mechanisms of the IGF-I osmoprotective effect.
The IGF-I osmoprotective effect was virtually eliminated by either, a blocking antibody to the type I IGF receptor. Hyperosmotic conditions caused cell cycle arrest of the cells as measured by the rate of 5H- thymidine incorporation and flow cytometry. Addition of 10M IGF-I protected the cells against hyperosmotic death without stimulating DNA synthesis. Programmed cell death (PCD) can occur by withdrawal of an essential external signal such as a growth factor. As determined by flow cytometry, several days of serum deprivation in isotonic medium did not precipitate PCD. In serum-free hyperosmotic media, PCD was initiated in 40% of cells in 24hrs and 80% in 72hrs. In hyperosmotic media containing 10M IGF-I, apoptosis occurred in only 12% of cells in 48hrs and 20% in 72hrs. Collectively, our results show that IGF-I acts as a neuronal osmoprotectant by protecting the cells against PCD.
These data suggest a potential therapeutic role for IGF-I in diabetic neuropathy or other conditions that lead to hyperosmotic states.

0704.19
EFFECTS OF NEUROTROPHIC FACTORS ON THE SURVIVAL OF SENSORY NEURONS FOLLOWING AXOTOMY IN THE NEONATAL MOUSE. L. L. Le, A.C. Lo, M. Lei, R.W. Oppenheim and J.J. Heumann. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157
Extensive neuronal death results from peripheral nerve section during the early period of postnatal development in mammals. Recently, it has been reported that a number of neurotrophic factors including BDNF, NT-3, NT-4/5, CNTF, IGF and GDNF promote the survival of axotomized motoneurons in vivo. The present study is aimed at determining whether these neurotrophic factors also rescue axotomized sensory neurons in the neonatal mouse. Our results show that approximately 25% of neurons in the lumbar spinal segment 4 dorsal root ganglion (DRG) died following sciatic nerve section in 5 day old mice, when examined one week after surgery. Several neurotrophic agents including NGF, BDNF, NT-3, CNTF, IGF-1 were able to promote the survival of axotomized DRG neurons. However, GDNF, previously shown to rescue axotomized motoneurons, was ineffective in rescuing sensory neurons from axotomy induced cell death. Our findings extend previous reports on the effects of these agents on survival of sensory neurons and suggest that some of these agents may be useful in the treatment of diseases and injuries affecting sensory neurons. Supported by Grants from NIH and MDA.

0704.20
GDNF AND BDNF PROTECT A CATECHOLAMINergic CELL LINE (CATH.a) FROM DOPEMIN INDUCED CELL DEATH. L. Gondi*, H. Bulaga, A. Adamu, R.J. Wyatt and J.M. Massarongo. Neuropsychiatry Branch, NINDS Neuroscience Center at St. Elizabeths, Washington, D.C. 20032. Giall cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) promotes neuronal survival of primary cultures. Therefore, we study the effects of growth factors on cell death. Dopamine (DA) (10 - 500 15) produces a dose dependent cell death (maximal 70%) in CATH.a cells. DA induced cell death is correlated with an increase in intracellular peroxides and is inhibited by catalase, suggesting that DA-induced cell death is caused by oxidative stress. Treatment of cultures with GDNF or BDNF reduced DA (100 15) induced cell death from 40% to 20%. Nerve growth factor, fibroblast growth factor and insulin-like growth factor-1 has been shown to have protective effect. These results indicate that GDNF and BDNF protect CATH.a cells from the oxidative stress of intracellular peroxides produced by dopamine autoxidation.
T05.1
GENESIS OF INTERNEURONS IN THE RAT STRIATUM. A.F. Sadikot*, R. Sasseville, S. Gauthier. Dept. of Neurology and Neurosurgery, McGill Neurological Institute, McGill University, Montreal, PQ, Canada.

The time of final mitosis (birthdate) of different classes of interneurons in the rat striatum was analyzed using the 5'-bromodeoxyuridine (BrdU) incorporation method. Double-labeling immunohistochemistry allowed simultaneous visualization of BrdU, and either calretinin, parvalbumin, choline acetyltransferase (ChAT), or somatostatin at the level of the precommissural dorsal striatum, most GABAergic interneurons expressing calretinin are born between E3-E4, whereas GABAergic interneurons expressing parvalbumin are born between E1-E2. As shown in studies using 3H-thymidine (Semba et al., 1988), our studies confirm that the birthdate of the main mass of cholinergic and somatostatinergic interneurons at this striatal level is between E3-E5 and E16, respectively. Thus, unlike the pauch and matrix projection neurons, whose peak birthdates are E13 and E18, respectively (van der Kooy and Fishell, 1987), GABAergic and somatostatinergic interneurons of the dorsal striatum leave the mitotic cycle at an intermediate time period.

Further studies are aimed at defining gradients of neurogenesis within the dorsal striatum, and at comparing birthdates of neurons generated in the dorsal or ventral striatum.

Supported by the MRC, RSQ, AANS.

T05.2

Molecular processes specific to motor neuron (MN) growth and development are poorly characterized. We are using clonal hybrid cells derived from embryonic mouse spinal cord MN which express typical traits of MN to identify previously uncharacterized genes expressed in MN. cDNAs preferentially expressed in hybrid MN were isolated by differential display reverse transcription polymerase chain reaction and differential screening. Eleven of 24 (42%) others had sequence homology to genes previously characterized from human, chicken or rat. Twelve (24%) others do not share identity with any currently known genes or proteins. One cDNA sequence was recently identified by others as a G protein β subunit gene. By northern blot we have identified an alternately spliced transcript of this gene which appears to be expressed only in brain and spinal cord. In conclusion, we have used hybrid MN cells to identify novel genes related to MN. One of these, a neural specific gene, appears to be present in MN. Twelve others appear to be novel genes that may have specificity for MN.

Supported by a grant from the Walter Broughton ALS Fund.

T05.3
FACTORS INFLUENCING THE DEVELOPMENT OF DENDRITE BUNDLES IN THE RAT'S SPINAL CORD. A. Gramsbergen, J. Eijlmeier-Paassening, F. Kok.

Medical Physiology, Groningen, NL-9712 KZ, The Netherlands.

Recently, we studied dendrite bundles in motorneuronal pools of muscles, which are involved in postnatal locomotion in the rat. Dendrite bundles specifically occur in pools of axial muscles and particular antigravity muscles in extremities. These bundles develop at the age when forelimb control positions changes to postural (from postnatal day 8 (P8) in the pool of the long back muscles and from P14 in the pool of the soleus muscle.

It is not known how this dendritic reorganisation is induced. Possiblities are: by supraspinal afferents; by muscle afferents or by retrograde influences from muscles via their motor nerves. We investigated the possibility of the latter by a total spinal transection at Th8-Th11 (N = 18) or a hemitranssection (N = 21). Muscle afferents were interrupted unilaterally at P10 by cutting the dorsal roots of L3-L5 (N = 21) and in another group (N = 18) we blocked neuromuscular transmission from P3-P12 in the soleus muscle by α-bungarotoxin (in silicon rubber). The bundle densities in the whole soleus muscle were labeled with Cholera Toxin subunit B at P18, 21P, and P30.

Results indicate that blocking neuromuscular transmission retards the development of dendrite bundles. While a total transection leads to a decrease in the number of dendrite bundles and dendrites per bundle (although the onset of this reorganisation is normal). Neither sectioning of dorsal roots, nor hemisection of the spinal cord has an effect on this development. It seems that both trophic influences from muscles and supraspinal influences are essential for dendritic reorganisation.

T05.4

During metamorphosis of the moth Manduca sexta, thoracic leg motor neurons (MN) persist from larva to adult but undergo dramatic morphological changes including reorganization of their central and peripheral processes. Previous studies have shown that these central and peripheral changes are under control of ecdysial hormones. Eleven of 24 (42%) others had sequence homology to genes previously characterized from human, chicken or rat. Twelve (24%) others do not share identity with any currently known genes or proteins. One cDNA sequence was recently identified by others as a G protein β subunit gene. By northern blot we have identified an alternately spliced transcript of this gene which appears to be expressed only in brain and spinal cord. In conclusion, we have used hybrid MN cells to identify novel genes related to MN. One of these, a neural specific gene, appears to be present in MN. Twelve others appear to be novel genes that may have specificity for MN.

Supported by a grant from the Walter Broughton ALS Fund.

T05.5

Due to size, human muscles have multiple endplate regions, and muscle fibers are usually associated with only one of these regions. Rat muscles, on the other hand, typically contain only one endplate region, and all of the fibers express a similar percentage of the muscle. There are four endplate regions in the guinea pig sternomastoid (SM) muscle, and all of the fibers in the SM muscle were labeled with Cholera Toxin subunit B at P18, 21P, and P30.

Results indicate that blocking neuromuscular transmission retards the development of dendrite bundles. While a total transection leads to a decrease in the number of dendrite bundles and dendrites per bundle (although the onset of this reorganisation is normal). Neither sectioning of dorsal roots, nor hemisection of the spinal cord has an effect on this development. It seems that both trophic influences from muscles and supraspinal influences are essential for dendritic reorganisation.

T05.6

There has been no systematic investigation of the spatiotemporal development of nuclei containing sensory (mesencephalic) neurons and motor neurons and their innervation of muscular muscle. The purpose of this study was to determine the timing of sensory and motor innervation of the masseter muscle and compare this process with the developmental stages of formation of mesencephalic and motor nuclei.

A total of 33 embryos representing gestational days 10.5, 11.5, 12.5, 13.5, 14.5, and 15.5 were obtained from intact CD-1 mice. Di-DiO was implanted into the embryonic masseter muscle or masseter region, into the mesencephalic mesencephalic region, or into maxillary or mandibular motor bud or tooth bud region, respectively. As the embryos were maintained at 38°C for 2-8 weeks to facilitate uptake of the dye, embedded in gelatin, sectioned at 85 µm using a vibratome, and examined under epifluorescence using a Zeiss microscope. The stains of labeled cells and their processes were acquired for analyses.

At gestation day 10.5, 4/4% of the cells with dye placed in the masseteric region demonstrated labeled cells in the trigeminal motor nucleus while 9% of the mesencephalic nuclei were labeled. Neurons in the trigeminal motor nucleus had no labeled cells which were not in the masseteric region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region. Labeled cells were found in the trigeminal motor nucleus, and not in any other region.
T05.7
EARLY BRAINSTEM-SPIXAL SEROTONERGIC AND NORADRENERGIC PROJECTIONS IN THE RAT EMBRYO: DOUBBLE LABELING STUDY USING DEXTRAN AMINES AND IMMUNOHISTOCHEMISTRY.
F. Auel* and R. Marchand. Centre de Recherche et Neurologie, Université Laval, Quebec, Canada, G1V 1U3
Previous studies done in our laboratory have described the location and distribution of the serotonergic and noradrenergic neurons projecting to the spinal cord early during the development of the rat embryo. At these stages, the rostral migratory stream (RMS) and the auditory structures are a longitudinal source of neuronal populations. This study is the first to identify the rostral migratory stream and the auditory structures as a longitudinal source of neuronal populations. The data shows that the RMS and the auditory structures are a longitudinal source of neuronal populations.

T05.8
SEROTONIN INCREASES THE EXCITABILITY OF EMBRYONIC CHICK MOTONEURONS RECORDERED FROM A SENSORY CORP SLICE PREPARATION.
L. Jasani*, B. Mendelsohn, K.D. Phelan and E. Garcia-Rill. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.
Intracellular recordings were obtained from motoneurons in an embryonic chick spinal cord slice preparation over a series of developmental stages (E12-E18). These time points bracket the critical period of development (E14) in which the ability of the spinal cord to recover from injury is greatest. The data shows that serotonin increases the excitability of motoneurons in the spinal cord slice preparation. This increase is mediated by the 5-HT3 receptor and can be blocked by the 5-HT3 antagonist ondansetron. The results suggest that serotonin may play a role in the development and maintenance of the excitability of motoneurons in the spinal cord.

T05.9
RELATIVE CONTRIBUTIONS OF POTASSIUM CONDUCTANCES AND SYNAPTIC INPUT IN DETERMINING INPUT RESISTANCE OF DEVELOPING MONOPODIAL MOTONEURONS OF THE MONODELPHIS DOMESTICA. J. LeBlond* and T. Cabana. Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Canada, H3C 3J7.
The extent to which potassium conductances and synaptic inputs contribute to the generation of the action potential is a fundamental property of excitable cells. In this study, we measured the contribution of potassium conductances and synaptic inputs to the generation of the action potential in developing motoneurons of the Monodelphis domestica. Our results show that potassium conductances and synaptic inputs contribute significantly to the generation of the action potential in developing motoneurons.

T05.10
The development of the spinal cord is a complex process that involves the formation of the neural tube and the development of the peripheral nervous system. In this study, we investigated the myelogenesis of the ventral (VR) and dorsal (DR) roots of spinal segments of the Monodelphis domestica. Our results show that the VR and DR roots develop independently and have distinct developmental pathways.
GROOMING DEVELOPMENT AND ACTIVATION IN WEAVER MUTANT MICE. V.J. Bolivar*, W. Danilchuk and J.G. Panksepp, Psychology Department, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1. 

Weaver (w/vw) mice have well specified nigrostriatal dopaminergic and serotinergic and after a 30 second swim period. Deficiencies in grooming of w/vw/wv pups are context and age dependent. Wv/wv spend less time grooming than controls; however, after day 13 postpartum during the post-swim period mutants attained grooming levels of pre-swim controls. Wv/wv pups also showed, from day 15, an increase in number of grooming bouts post-swim. This reflects previous activation by swimming. Our data suggest activational effects can be separated from balance, postural or sequencing problems. Although controls displayed longer bouts than mutants overall, during the post-swim period (day 13 and older) w/vw wv pups produced bouts as long as pre-swim controls. Strokes used by w/vw/wv pups tended to cluster around the early grooming sequence phase and some later strokes were never used by the mutants. These results indicate the importance of examining behavioral ontogeny under different conditions, as well as the value of using multiple measures when examining action sequences. Mutant mouse research holds much promise in linking cellular, circuit and behavioral levels of organization.

FACTORS RESPONSIBLE FOR POOR FUNCTIONAL RECOVERY AFTER PERIPHERAL NERVE LESIONS. Neil Tyerman, Mukalla Bai and Tessa Gordon*, Department of Pharmacology and Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2Z2. 

Even primary nerve repair may be associated with minimal functional recovery when long delays are incurred between the peripheral nerve injury and target reinnervation. In this study, we have systematically and independently controlled the duration of axotomy (X), duration of the distal nerve sheaths (SH) and muscle denervation (M) to determine the relative contribution of each factor to functional recovery. In rats, the posterior tibial nerve (TIB) was cut and sutured to the CP nerve via a contralateral common peroneal (CP) nerve graft (10-15mm) to reinnervate tibialis anterior (TA) muscle, either immediately or after a 10-15mm TA graft, or TA denervation. At least 6 months after graft surgery, number and size of TA motor units were determined as measures of number of regenerated nerves and numbers of muscle fibers per motor unit. An average of 99±5.3 motorneurons innervated TA after immediate grafting, in all cases where nerve repair was delayed, the number decreased as a function of delay between X, SH or D and nerve repair, reaching an asymptotic value of 30.2±3.6, 41.4±5.4 and 13.7±3.4 at 300 days.

In the light of our previous findings that deterioration of intramuscular nerve sheaths is the primary factor limiting reinnervation of long-term denervated muscle (Fu & Gordon, J. Neuroscience, in press), these findings indicate that the growth response of AX motoneurons and the growth environment of denervated nerve sheaths deteriorates with time. The better recovery after prolonged graft denervation than after prolonged muscle denervation indicates that migration of Schwann cells from the 'fresh' nerves into the 'old graft' may have facilitated regeneration. We are presently evaluating the molecular basis for the reduced growth response of long-term denervated nerves and denervated nerve sheaths (see You et al. abstract).

STUDIES ON SLOW AND FAST COMPONENTS OF AXONAL TRANSPORT IN MICE LACKING THE NEUROFILAMENT LIGHT (NFL) GENE. S. Cuilliard-Deneps, Q. Zha, D. F. Playford* and J.P. Julien, Centre for research in Neuroscience of McGill University, The Montreal General Hospital Research Institute, 1650 Cedar ave., Montreal, Quebec, Canada, H3G 1A4. 

The neurofilament triplet proteins form the 10 nm filaments found in almost all neuronal cells and are the major constituents of the cytoskeleton in these cells. The presence of the three components (neurofilament light, medium and heavy subunits: NFL, NFM and NFH) is required for a proper polymerization. Our laboratory recently produced a mouse line lacking the NFL gene. In the absence of NFL, expression of the two other subunits, NFM and NFH, in the nervous system is down regulated. Our results reveal the existence of a compensatory mechanism resulting in up-regulation of tubulin and microtubule-associated proteins. To study axonal transport, [S-35]methionine was injected in the ventral horn of the spinal cord (between L2 and L3) of low- and control mice. The mice were sacrificed after different time intervals following injection. The sciatic nerves were dissected and divided into 3 mm segments. Proteins were extracted from each segment, fractionated on SDS-PAGE, and analyzed by fluorography. Our preliminary results reveal a dramatic increase in the rate of axonal transport of tubulin in the NFL knock-out mice.

SNAP-25 ISOFORM mRNA EXPRESSION IN SPINAL MOTOR NEURONS AFTER NERVE INJURY. G. Jacobsson, F. Pfeiffer*, X. Zhang, I.C. Bank and B. Meister, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, and Department of Developmental Biology, Uppsala University, Sweden. 

Synaptosomal-associated protein of 25 kDa (SNAP-25) is a protein involved in the molecular regulation of exocytosis. SNAP-25 is located at the presynaptic plasma membrane and is a component of the 25S multi-subunit complex that mediates vesicle docking and fusion. The protein exists in two isoforms, which arise from alternative splicing of exon 5. In order to study the plasticity in SNAP-25 gene expression, we have axotomized the sciatic nerve and studied SNAP-25 mRNA levels in spinal motor neurons using in situ hybridization and isomorph-specific oligonucleotide probes. In all animals, SNAP-25a mRNA was detected in the nucleus of motorneurons, whereas SNAP-25b mRNA was present in the cytoplasm. After axotomy, the levels of SNAP-25a and SNAP-25b mRNA decreased in motorneurons belonging to the sciatic pool. Measurements of the grain density over cells on the lesioned side as compared to the unlesioned side revealed a significant decrease after 2 days. The maximum decrease was detected at 7 days (62% for SNAP-25a and 67% for SNAP-25b), while levels had slightly recovered by 14 and 28 days. Ventral root avulsion produced a similar decrease in SNAP-25 mRNA levels in lesioned cells, indicating that the decrease in SNAP-25 mRNA is due to the axonal severance in itself. The results suggest that SNAP-25a and SNAP-25b mRNA have different localization within the cell and that SNAP-25a mRNA is down-regulated in specific populations of spinal motorneurons after nerve injury.

ACCUMULATION OF NEUROFILAMENTS CAUSES DENDRITIC ATTRACTION IN MOTOR NEURONS. J.-M. Kong, D.W. Cleveland*, and Z.-L. Xie*, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; *Ludwig Institute for Cancer Research and Department of Neuroscience, UCSD, La Jolla, CA 92093.

Accumulation of neurofilaments (NFs) in motor neurons has been widely observed in human motor neuron disease, of which the most common forms are amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). In transgenic mice, severe accumulation of NFs induced by forced over-expression of either NF-L or NF-H leads to loss of motor function, resulting in paralysis, skeletal muscle atrophy, and finally death (Xu et al., 1993, Cote et al., 1993). To further understand how NF accumulation causes motor neuron dysfunction, we have examined the structure of neuromuscular junction by EM and motor neuron morphology by Golgi staining in the NF-L transgenic mice. Except for the increased NF content in nerve terminals, the neuromuscular junction remains intact. However, a quantitative measurement of the spinal cord motor neurons reveals a significant shortening in the length of their dendrites (by more than two fold in comparison with the wild type), leading to a reduced density of dendrites, a shrunken area coverage by the dendrites, and a loss of distal dendrites. These changes could cause a reduction in the number of synapses contacting the motor neurons and consequently lead to motor neuron dysfunction.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
LAMINAR DISTRIBUTION OF GLUTAMATE RECEPTORS IN DEVELOPING RAT VISUAL CORTEX. B. Gordon*, L.N. Fu and T. LeDoux, Dept. of Neurosciences, Univ. of Oregon, Eugene, OR 97403.

We studied the development of glutamate binding sites in the visual cortex of Long-Evans rats. We used quantitative autoradiography to describe the development of [3H]MK-801 binding sites (an open channel ligand for NMDA receptors), [3H]AMPA binding sites, and [3H]MHamm binding sites. As a non-competitive control we used [3H]muscimol, a ligand for GABA receptors. Binding of each ligand was measured at 14, 18, 26, 39 days (30 weeks). At 14 days all binding sites were of similar magnitude across all layers, with a slight peak in layers 2/3 and 4. In older animals [3H]MK-801 binding was higher in layers 2/3 and 3/4 in the deeper layers. Layers 5 and 6 were lower than in all and 39d animals similar to 18d, except that in the thalamocortical connections [3H]MK-801 binding was much lower than in layers 2/3 and 4. These results suggest that NMDA receptors are involved in plasticity in the rat. AMPA binding sites may also be involved. The precise role of these binding sites requires further study. The present results in the rat contrast somewhat with results we reported previously for cat (cf. Gordon et al., 1994). (Supported by NEI grant EY04050 to BG).

REGIONAL AND LAMINAR CHANGES IN SYNAPTIC LOCALIZATION OF NMDA RECEPTOR SUBUNIT SPlice VARIABLE NR1 IN RAT VISUAL CORTEX AND SPINAL CORD. R.R. Johnson, X.F. Jiang, and A. Buchacher, Dept. Anatomy & Neurobiology, Wash. Univ. School of Medicine, St. Louis, MO 63110.

Regional and laminar distribution of the NR1 subunit of the NMDA receptor was examined at the light (LM) and electron microscopical (EM) levels using a new antiserum directed against a differentially spliced C-terminal exon (NR1-C1; Sheng et al., 1994). This exon is expressed in a restricted number of NR1 splice variants (Sugihara et al., 1998, Holmes et al., 1999). NR1-C1 is a major substrate for posttranslational modification by NMDA receptor-dependent phosphorylation (Tinglis et al., 1993). The most striking result was that the pattern of NR1-C1 immunoreactivity in both the hippocampus and visual cortex was more restricted than that previously shown using other NR1-specific antibodies (Hunsley et al., 1994; Peralta et al., 1994; Siegel et al., 1996), and did not label cells in CA3, dentate gyrus and subicular regions of the hippocampus and in layer 4 of the visual cortex. Regional and laminar differences in synaptic NR1-C1localization were confirmed by quantitative EM.

Surprisingly, in addition to abundant postsynaptic staining, immunoreactivity was found in >90% of all terminals in the dorsal subiculum, but in only a small percentage (<1%) of terminals in visual cortex. This strongly suggests that presynaptic NMDA receptors play a major role in neuronal processing of hippocampal output through the subiculum, but be a relatively minor role in V1.

In sharp contrast to the pattern in the adult, there was relatively little regional and laminar variation in NR1-C1 immunoreactivity in the developing brain (PND 11). Interestingly, NR1-C1 splice variants were present in cortical layer 4 as well as in CA3, dentate gyrus, and subiculum at the early time point but were largely excluded from these structures in the adult. This suggests that NR1-C1 splice variants may play a role in determining the critical period for visual cortical layer 4 - the expression of expression within upper and lower layers of the cortex and in CA1 of the hippocampus in the adult could provide a substrate for plasticity in cortico-cortical connections and Schaeffer collateral synapses beyond the critical period.

Developmental expression of the immediate early gene Egr-1 mirrors the critical period in cat visual cortex. L.V. Kaplan, Y. Gao, K. Kaelberer, and A. Mian. Deparment of Neurosciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

The aim of this study was to determine the postnatal developmental profile of Egr-1 protein expression across the layers of cat visual cortex and relate it to the state of visual cortical development and plasticity. Cats at various postnatal ages (P2, P8, P15, P21) were used. Coronal sections from visual cortex were processed for immunohistochemistry with avidin-biotin visualization. In young animals (<3 weeks), Egr-1 positive cells were restricted to deep cortical layers (layer V/Subplate). With increasing age, Egr-1 immunoreactivity spread across the layers of visual cortex in inside-outside manner, and by 5 weeks of age, Egr-1 protein was expressed in all layers of the visual cortex. In addition, an intensely stained band of labeled cells in the lowest portion of layer IV appeared at 1 week of age and was present in all older animals. Egr-1 expression remained high until approximately 10 weeks of age and then gradually began to decline in layer IV with little change in supragranular and infragranular layers. Therefore, in adulthood, Egr-1 positive cells were located preferentially in the layers above and below layer IV.

This pattern of Egr-1 expression in developing cat visual cortex exhibits both similarities and differences compared to that of different visual cortical areas. With the development of orientation selective receptive field properties, and with the level of visual cortical plasticity, suggesting an involvement of Egr-1 expression in these processes.

DIFFERENTIAL DISTRIBUTION OF TWO GLUTAMATE DECARBOXYLASES (GAD67 AND GAD65) IN THE NEWBORN AND ADULT CAT VISUAL CORTEX. Y. Guo, L.V. Kaplan, N.E. Cooper and G.D. Mower*, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

The identification of two forms of glutamate decarboxylase (GAD67, GAD65), the synthetic enzyme for GABA, raises the possibility that they may play different roles in the development of various GABAergic circuitry. We have used antibodies to compare GAD67, GAD65, and GABA positive neurons in neonatal (4-6 days) and adult cat visual cortex. In adults, GABAergic neurons were found in all cortical layers but little cell body staining was seen with the antibody to GAD65. Higher power observation revealed a complementary intracellular distribution of GAD67 and GAD65, with GAD65 being present mainly in cell bodies and GAD67 in the terminal axonal arborizations. In newborns, GABA and GAD67 were present in two distinct bands, one superficial (Layer I), another deep (Layer V/Subplate). Unlike in adults, GAD67 positive cell bodies were clearly evident in neonates and distributed similarly to, but less frequently than, GABA and GAD67. Higher power observation again revealed that the density of GAD67-positive punctate structures was higher than that of GAD67 punctate structures. The distinct intracortical localization of the two isoforms in adult cat visual cortex is consistent with the notion that GAD67 (holoGAD) provides the basal pool of GABA and GAD65 (apoGAD) is specialized to respond to short-term increases in demand for GABA in axon terminals. That the intracortical and laminar distribution of both isoforms of GAD and of GABA differs between neonatal and adult VC suggests that these two forms of GAD may be differentially involved in two distinct aspects of postnatal maturation of visual cortical GABA circuitry, dying off of embryonic cells and differentiation of the adult GABA system.

LAMINAR PATTERNS OF GABA RECEPTOR EXPRESSION IN FERRET PRIMARY VISUAL CORTEX ARE ESTABLISHED PRIOR TO CORTICAL INVASION BY GENICULATE AFFERENTS. A. Smith and J.D. Thompson, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Quantitative in vitro autoradiography has been employed to monitor the expression patterns of GABA receptor proteins in the primary visual cortex of ferrets throughout postnatal development. [3H]N-methyl-[3H]GABA, a receptor agonist, and [3H]muscimol, an agonist at the benzodiazepine modulatory site, were used to label GABAergic neurons. Proteolipid primary visual cortex was identified by transneuronal antipomial staining of geniculate axon terminals using intracortical injections of wheatgerm agglutinin conjugated to horseradish peroxidase. Perikarya and dendrites of the cortical plate was monitored by neuronal biotidation using 5-diamino-2- deoxyuridine immunocytochemistry.

GABA receptor density was highest in the primary visual cortex doubles in the first postnatal week, after which time layer-specific laminar distribution patterns are observed. From postnatal day 8 (P8), when migrating layer IV neurons are arriving in the cortical plate, GABA receptor density in layer 4 exceeds that in the infragranular layers or layers III/IV (P<0.001). GABA receptor protein expression in all layers continues to increase throughout development, increases in layer I and IV preceding increases in other layers reaching a maximum a month after eye opening and then decreasing slightly after P65. Unlike EAA receptors (Smith & Thompson, J. Physiol. 486.BP) changes in GABA, receptors may be brought about by changes in the state of the geniculate fibres and preliminary evidence indicates that disruption of the geniculocortical pathway does not affect the early patterning of GABA, receptor expression.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
706.7
CHANGING DISTRIBUTION OF NMDA IN THE DEVELOPING VISUAL
CORTEX. S. M. Cashman*, C. K. Chang and C. J. Stagni. HMBI and Dept. of
Molecular and Cell Biology, University of California, Berkeley, CA 94720.
NMDA receptors are thought to play an essential role in the
development of the visual cortex. We have examined the distribution of the
NMDA1 receptor subunit protein in the ferret using 3 different antibodies: two
are raised against overlapping, C-terminal, alternatively spliced domains (AB1
& 2), and the third was raised against a non-alternative spliced domain
(mA3B). At embryonic day 35 (birth = E41), all 3 antibodies stain fibers within
marginal zone (M), intermediate zone (I), and the outer cortical plate, and subplate
neurons (located within future white matter); the subplate neurons are stained
most intensely. There was no detectable immunostaining of migrating cells in the
intermediate zone (I), while layer 6 stain as intensely as the subplate
neurons. In addition mA3B, but not the other antibodies, stains a band of
horizontally orientated cells within the intermediate zone. By the 7th postnatal
week, when ocular dominance columns have begun to form in cortical layer 4,
staining of layer 1 and the white matter (subplate neurons) is undetectable with
call 3 antibodies. Antibodies raised against a non-alternative spliced domain
mRNA (mA3B). Antibodies raised against a non-alternative spliced domain
mRNA (mA3B). In addition mA3B, but not the other antibodies, stains a band of
horizontally orientated cells within the intermediate zone. By the 7th postnatal
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mRNA (mA3B). Antibodies raised against a non-alternative spliced domain
mRNA (mA3B).

706.9
SYNAPtic VEsicle PROtein IMMUNoreactivity DURING DEVELOPMENT IN MACAQUE MONKEY PRIMARY
VISUAL CORTEX. T. Yoshikawa & S.H.C. Hendry, Krieger Mind/Brain Inst. & Dept. of
Neuroscience, Johns Hopkins University, Baltimore, MD 21218.
Changes in synaptic activity are essential in the functional and
anatomical development of mammalian neocortex. Such synaptic activity
is influenced by intrinsic and extrinsic neuronal input in different
dimensional solutions during development. To assess the pattern of
laminar-specific events in the development of synapses in macaque primary
visual cortex (V1), we have examined the expression of synaptophysin and
electron microscope antigenic proteins in developing visual cortices in the
fetal and infant macaques. At embryonic day E15 (gestation = 165 days),
immunostaining of synaptophysin revealed distinct laminar boundaries
with intense punctate immunoreactivity in layers 4A, 4C, and the bottom of 4C5
through 5. Immunostaining in layer 4A showed clear evidence of a honeycomb or lattice, even though no
pattern was evident in the pale cortex. The emergence of layering at
layer 4A at this age. A similar irregular honeycomb pattern was also
evident in deep layer 5. The laminar pattern seen at E15 continued to be
visible in later age, but became less laminar-specific
immunostaining in postnatal tissues. These observations suggest that the
maturation of synapses occurs in a laminar specific manner in
macaque V1. (Supported by NIH grant 004532. We thank Dr.
Anita Hendrickson for providing fetal and neonatal tissue.)

706.11
DEVELOPMENTAL CHANGES IN EVOKED SPATIAL ACTIVITY
Morinobu Wakamatsu, Akiatug-shi, Kagawa 724-01, Japan. *Physiol. Lab, Univ.
Cambridge, U.K.
Spontaneous synchronized activity is believed to play a role in the refinement of
correlate with the developing nervous system. Developmental activity changes in
dissociated cortical cultures have been described previously (Charlety et al., Soc.
Neurosci. 1994, 334,9). Here we have mapped developmental changes in the
effects of spatially synchronized activity in vertically
oriented cortical slice. P1 rat cortical slices were fused by
gentle centrifugation onto substrates containing an array of 464 embedded electrodes, and
and . After 5 days in vitro (DIV), spontaneous activity was
antibodies to subunit proteins of the NMDA receptor were
layered at various stages of development. The results suggest that, like
dissociated cultures of cortical neurons (Robinson et al., 1995).
J. Neurophysiol. 78, 759-769., we have the capacity for spontaneous activity of
low-voltage-synchronized bursting activity, that the pattern of propagation is determined by
the anisotropic structure of the local circuitry of the cortex, and that there is
progressive sharpening in the local synchronization of bursting with development.

706.8
SUBCELLULAR REDISTRIBUTION OF NMDA RECEPTORS IN DEVELOPING VISUAL CORTEX OF CATS. C.G. Gro*,
for Neural Sci., New York Univ., NYC, NY 10003 & Dept. of
Neurosc., The Johns Hopkins Univ. Med. Sch., Baltimore, MD.
Electrophysiological studies have shown that, during the
postnatal period spanning the critical period, NMDA splice domain
in the visual cortex undergo alterations in their laminar distribution and are more
sensitive to blockade by NMDA-receptor antagonists. Using a previously
described antisense directed against the NMDA-R1 subunit, we determined whether these pharmacological changes correlate with changes
in the cellular and subcellular distributions of the receptor. During the first
three postnatal weeks, light microscopy showed prominent staining of a
subset of neurons that were scattered throughout the cortical thickness, including layer 6B. These neurons exhibited non-pyramidal
perikarya and variable dendritic processes which were studied with
immunocytochemische techniques. In addition, fine beaded processes,
most likely axonal, were prominent in the white matter. In contrast, tissue
obtained from older animals exhibited a more uniform distribution of
punctate labeling in the neuropil. Electron microscopy revealed that these consist of immunoreactivity in small to medium
length axons, dendrites, and terminals. These results indicate that the NMDA-R1
subunits undergoes a dramatic redistribution during the critical period for
developmental plasticity. Supported by NEI-EY00555, NSF Presidential
Faculty Fellows RCD 95-53750, NINDS NS 39044-01 & HSPG RG-1695.

706.10
SPONTANEOUS ACTIVITY OF NEURONS IN ORGANOTYPIC CULTURES OF RAT NEOCORTEX DURING DEVELOPMENT IN VITRO.
D.E. Charlety* & A. Alisa. Neurobiology Group, Max Planck Institute for
Biophysical Chemistry, 37071 Gottingen, FRG.
Neurons in organotypic cultures (OTCs) of rat cortex may develop highly
dependent firing patterns, including stereotyped burst pattern 1. We wondered
whether in neocortical OTCs spontaneous bursting activity displays a progressive
time-dependent onset for 1 neurons in OTCs of
hypocampal. Action potentials were recorded extracellularly from 64 neurons in 102 OTCs (from 3 - 9 days old) using the intracellular technique of
imprinting. Neurons divided operationally into 4 classes: 1) spontaneously active (nonburst); 2) Spontaneously active, bursting (SpB); 3) Spontaneously active, mixed (SpM) neurons, i.e. with both SpB and SpM activity. In OTCs 1 and 2 and all activity classes were present. The proportions of activity classes changed during development in vitro. From the first to the second week the proportions of SpM neurons decreased (from about 60% to about 30%) and that of mature active classes increased accordingly. Furthermore, no systematic change occurred until the end of the 2nd month. During development in vitro a slight (not significant) upward trend in average firing rate, interspike firing rate and burst duration was found. A temporal and
analysis of firing patterns by means of double recordings revealed a high degree of burst synchrony across OTCs with multiple sources for triggering individual
bursts. A progressive time dependent onset of bursting activity was not seen. Rather it was found that SpB neurons, in particular those displaying longer lasting bursts, were present from the 4th day on and in OTCs treated with more than average mechanical
manipulation during preparation.
(1989),Supported by A.v.Humboldt SFAK 0023 and Gobalti Villa BEU 04.066.

706.12
DEVELOPMENT OF CORTICOCOLICULAR CELLS IN NORMAL AND ANOPHALMIC MICE. M. KHACHAB* & L. L. BRUCE. Dept.
Biomed. Sci., Creighton University, Omaha, NE 68178.
Anophthalmic corticocollicular (CC) axons show a delayed growth into the
superior colliculus (SC) when compared to normal CC axons. This delay may be due to a delayed development of the anophthalmic visual cortex or the absence of retinocollicular (RC) axons (Khachab and Bruce, Soc.
Neurosci. Abstr. 1994). To test these possibilities, the development of
anophthalamic CC neurons was compared to that of normal CC neurons. Injections of the fluorescent dye, DIL, were made in the SC of normal
(C57BL/6J) and anophthalamic (129SV/C57BP) embryonic and postnatal mice
to retrogradely label the CC neurons. In normal mice, labeled cells were first observed at E15. Thus, these mice had a short apical dendrite that had not yet
extended to the superficial layers. By P0, the apical dendrites extended to
the superficial layers and I or 2 basal dendrites were present with no
collaterals. At P2, the basal dendrites had multiple branches and most apical
dendrites were bifurcated. At P4 and P6, the main shaft of the apical
dendrites ramified in the superficial layers forming an elaborate terminal tuft
and the basal dendritic tree continued to arborize. In contrast, cells in
anophthalamic mice were less mature at comparable ages. By P0, only a few
dendrites and a fine axon were present. By P4, some apical dendrites had bifurcated and all appeared to extend to the superficial layers with a fine
terminal tuft. Basal and apical dendrites had relatively few branches and
dendritic collaterals. Thus, a delay in the maturation of the anophthalamic CC neurons appears to be responsible for the delayed growth of the anophthalamic CC axons rather than the absence of the RC axons.

The visual cortex of the cat develops many of its physiological and anatomical characteristics after birth. However, there are few studies on dendritic development in the visual cortex. We have studied the morphological development of the dendrites of neurons of layer 4 stellate cells. These cells are the primary recipient of visual information from the lateral geniculate nucleus. We intracellularly injected individual neurons with biotinylated Lcflcer Yellow within 300 microns fixed cortical sections and processed the sections by the ABC method. Layer 4 stellate neurons were imaged and analyzed in 3 dimensions using Sholl concentric rings. In newborn kittens these cells already had most or all of their primary dendrites and fairly extensive dendritic branching. Branching increased substantially within the first week and only slightly over the next 3 weeks. Dendrites grew in length for at least the first 4 weeks. Additional to dendritic length were proximal as well as distal since the length of first order branches increased. These results demonstrate that dendritic development is coincident with the period of synaptogenesis and most probably reflects that of sensitivity to experience dependent modification. Supported by NSF grant IBN9221246 to SBT.

NEURONS IN THE STRIATE CORTEX OF FOUR-WEEK POSTNATAL KITTENS EXHIBIT ADULT-LIKE INHIBITORY PROPERTIES. E.S. Green, G.C. D’Angelo, C. DeAngelis, and E.S. DeAngelis. Brain Research, University of California, Berkeley, California 94720-2020.

Excitatory characteristics of neurons in the kitten’s visual cortex are thought to be largely mature by four weeks postnatal (Mitchell and Timney, 1984.) However, the status of inhibitory properties at this age is not clear. We have studied specifically the properties of end- and side-inhibition and cross-orientation suppression using single cell recordings in the visual cortex of four-week kittens and compared our findings with data from adults. Responses were recorded extracellularly from neurons in area 17 of paralyzed and anesthetized kittens. To elicit excitation, we used drifting sinusoidal bars of varying parameters. To test for end- and side-inhibition we varied the length and width of the excitatory grating. The tuning properties of end- and side-inhibition were studied by varying the orientation and spatial frequency of patches located outside of the excitatory receptive field. To measure cross-orientation suppression the excitatory grating was overlaid with a drifting grating of orthogonal orientation.

Our results show that all three types of inhibition are present in neurons of kittens at four weeks postnatal. As in adults, every cell tested in the kittens exhibited cross-orientation suppression. Maximal cross-orientation suppression could be elicited from a region of approximately the same size as the classical receptive field. Neurons showing end- and side-inhibition in a similar degree, neurons showing only end-inhibition, and neurons showing only side-inhibition were also present in kittens, and occurred in roughly the same proportions as in cats. Finally, end- and side-inhibition were well-tuned for orientation and broadly tuned for spatial frequency, as in adults. These experiments show that intraretinal inhibitory mechanisms are quite mature by four weeks postnatal and suggest that inhibitory and excitatory processes develop in parallel. (EY01175)

RECIPIROCAL LAMINAR LOCALIZATION AND DEVELOPMENTAL REGULATION OF BDNF AND NT-4 mRNA DURING VISUAL CORTEX DEVELOPMENT. E. Latif, A. Hokfelt, and J.L. Shatz. HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The segregation of thalamocortical afferents into occipital dominance columns (ODCs) that occurs during visual system development in higher mammals is an activity-dependent process in which inputs from the two eyes compete for synaptically specialized layers of visual cortex. In this process, which involves the selective growth and retraction of axonal branches, competition for a trichrome substance released by the target neurons. A growing body of evidence implicates the family of neurotrophins in this process and other forms of synaptic plasticity. A potentialmediator of this activity is the neurotrophin, BDNF and NT-3, was assessed using in situ hybridization to localize gene expression in cat visual cortex during postnatal development, and after the period for segregation of thalamocortical afferents in layer IV. The expression patterns of these two neurotrophins were found to be reciprocal, both temporally and in terms of cellular localization. BDNF mRNA expression is hardly detectable in visual cortex soon after birth (P7), but increases dramatically after eye opening (P15), where it is found to be expressed in large neurons in deep cortical layers. By the height of the critical period (P28), the pattern is similar to that in the adult, with high levels in large neurons in both deep (V5) and superficial (V1) layers. NT-3 expression is upregulated at high levels in visual cortex prior to the onset of the critical period (P7), mainly in small, presumably stellate neurons in the deep portion of layer IV (V). This cortical expression stage declines and is almost undetectable by the end of the critical period (P45). In neither case was glial neurotrophin expression observed.

This dynamic pattern of neurotrophin expression during the critical period for thalamocortical segregation suggests a role for these factors in the selective growth and stabilization of eye-specific connections during ODC formation. Supported by NSF predoctoral fellowship (EL) and NIH grant E040858 (AH).
GITTER CELLS IN DEVELOPING CORTICAL WHITE MATTER ARE INVOLVED IN AXON PHAGOCYTOSIS AND TRANSFORM TO RESTING MICROGLIA: a study of activity-dependent receptorization giving rise to occular dominance and orientation maps. The adaptive changes include axon and synapse elimination of such an extent that the number of microglial cells may rise in the phagocytic capacity of giant cells. We tested this hypothesis by injecting the leptomeningeal fluid into the leptomeningeal glial nuclei of kittens aged between 2 - 3 months and recording transcellular and extracellular signals due to phagocytosis. (Thannat et al., TINS 17:177, 1994) after survival times of one to 12 weeks. Fluorescence microscopy revealed DII staining at three distinct locations: i. intradermal layer, ii. a band of perivascular label in layer IV, and iii. a distinct population of small cells in the white matter (WM) underlying the visual cortex. Double-labeling experiments with the astroglial marker GFAP and the microglial/macrophage marker GSA-I-B4 identified these cells as 'gitter' cells. Transcellular staining in WM was only observed when the dye injection was performed prior to the third postnatal week, coincident with the transient presence of 'gitter' cells up to that age. No transcellular staining of giant cells was observed in the cortic gray matter. The failure of gitter cells was assessed by GSA-I-B staining 10 weeks after D II staining performed at an age of two weeks. Cells in the WM, transcellularly stained by the early DII injection, now revealed the typical ramified morphology of resting microglial cells. We conclude that 'gitter' cells are involved in the phagocytosis of neuronal structures in early postnatal development and later transformed into astrocyte. The failure to observe transcellular labeling of giant elements during the period for activity-dependent plasticity challenges a phagocytotic role of glia as a mechanism of regressive plasticity, i.e. axonal pruning. (Supported by a NSHT project)

AXONAL REGENERATION III

T07.3
THE CRITICAL PERIOD FOR GROWTH OF DORSAL SPINOCELLULAR AXONS THROUGH A LESION OF THEIR SPINAL PATHWAY STUDIES USING THE NORTH AMERICAN CROW (COCCHELLO, M., Field, and R. T. Kupferberg. Mel thebein, Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.)

Opsums have been at focal stages of development, 12-12 days after conception, making it possible to manipulate them experimentally without infringing surgery. We have taken advantage of the opportunity to show that some dorsal spinal topocellular neurons (DSTC) are able to grow through a lesion of their spinal pathway from postnatal day (PD) 5-9 (Terman et al., 1994). Such growth does not occur in adult opsums (unpublished observations), but with this critical period has not been determined. In order to address this issue, opsum pups ranging in age from PD1-3 days 8 and still attached to the nipple were anesthetized so that their spinal cord could be hemised at 18 or 19. They were maintained with their mothers for being subjected to bilateral injection of Fast Blue or Fluoro-Gold into anterior lateral portion of the cerebellum, the major target of DSTC axons. The site of the injections was to label any neurons in Clarke's nucleus (CN), caudal and ipsilateral to the lesion, that supported axons which reached the cerebellum. In the opsum, as in other species, DSTC axons originate within CN and project ipsilaterally to the cerebellum (unpublished results). After survival days 7, the pups were reanesthetized and perfused transcardially with saline and then with formalin so that the brain was decalcified transversely, and examined with a fluorescent microscope. In all cases, neurons in CN central to the lesion were labeled bilaterally in roughly equal numbers. Labeling in CN caudal to the lesion was not observed in any case. This implies the P13 DSTC axons do not grow through or around a lesion of their spinal pathway to innervate the cerebellum. In contrast, at least some brodmann spinal axons grow through or around a comparable lesion (Lee et al., 1994). Our results suggest that the critical period for growth of DSTC axons through a lesion of their spinal pathway ends considerably earlier than that for comparable growth of brainstem axons (Supported by NS-25095 and 10105).
T07.5
REGISTRATION OF THE VESTIBULAR NERVE IN CATS. G. L., J. Eldan, A. Newman, J. Lopez, and V. Horoaga. Otolaryngology, Hadassah University Hospital, Jerusalem, Israel; Victor Goodhill Ear Center, UCLA Sch. of Med., Los Angeles, CA, 90024

The purpose of this study was to examine the vestibular nerve regeneration in cats. Under general anesthesia and in aseptic conditions, the cats (n=3) were subjected to a VIIth nerve (vestibular part) neuropathy on both sides by subcutaneous approach. The cats were allowed to survive up to a maximum of 6 months. Short latency vestibular evoked potentials (VEMP) were recorded in response to angular acceleration impulses. Auditory nerve-brainstem response (ABR) was also used for monitoring the cochlear function. Following the nerve transection, the VEMP disappeared immediately. At two months post transection time (PTT), the VEMP reappeared with P1 and P2 waves of longer latencies and lower amplitudes. Four months PTT, P1-P2 waves were clearly recognized with P1-P2 latencies recovered to normal range. At the 6th month PTT, the amplitudes of the P1-P2 waves dramatically increased and the VEMPs had an appearance close to that observed before the transection. On the other hand, ABR showed that the cochlear nerve was partially transected. No ABR recovery was observed until the 6th month PTT. Histological examination indicated that the Scarp's ganglion showed full complement of ganglion cells and the dendrites were identified projecting to the crista and maculae which were in normal morphology. In the central portion of the vestibular nerve and the brainstem, the nerve fibers had lost their orderly pattern. The thicker and thinner fibers intermingled each other. Protargol stained specimens demonstrated that these fibers were long, tortuous and somewhat unusual trajectories comparing to control. However, these fibers finally projected to the neurons in all the vestibular nuclei. Degeneration was observed in the partially transected cochlear nerve. Therefore, the histological results supported the physiological findings (ABR and VEMP) following the nerve transections. The present study suggests that the vestibular nerve is able to regenerate following the axon transection in cats. (Supported by Oberkotter Foundation, U-5-Israel Bilateral Foundation No.89-00191, and NICDC grant DC01440)

T07.6

Unilateral electrolytic lesions of the entorhinal cortex result in deafferentation of ipsilateral dentate granule cells. Recovery in this deafferentation model has been suggested to be due to terminal proliferation and synapse formation of the crossed temporalis pathway. However, enhanced electrophysiological activation of granule cells by the crossed temporalis a few post-lesion months following the lesion, preceding anatomical correlates of synaptogenesis. Thus, early recovery may result from either changes in granule cell dendrites (such as sprouting or atrophy) or synaptic terminals rather than regeneration. We investigated dendritic alterations in granule cells (6-8 week male F344 rats) in vivo at ten days post-lesion by using intracellular neurobiotin staining. Adequately labeled cells were reconstructed on a computerized 3D microscope (Neurolucida). Normal granule cells showed typical unipolar architecture, branches reaching the hippocampal fissure and a total dendritic length of 3.55 ± 0.44 X 106 µm² (n=10), in agreement with previous data. Preliminary results at ten days following unilateral entorhinal cortex lesions show a total dendritic length of 2.43 X 106 µm². The average width of the entire molecular layer was reduced by 14% in lesioned animals to 189 ± 6.4 µm compared to 220 ± 8.5 µm in normal animals. Morphologically, granule cell dendrites in the outer molecular layer of lesioned animals appeared tortuous as they extended to the hippocampal fissure, possibly due to shrinkage. These results indicate that loss of afferent pathway may lead to shrinkage of the molecular layer and possibly secondary dendritic alterations in granule cells. This format of analysis may provide a method to assess both the physiological and anatomical aspects of early functional recovery following the pertinent path lesion. Supported by NHM (KBR), NIA (GKP), NINDS (DAT) and VAMC (DAT).

T07.7

The internuclear neurons of the cat abducens nucleus project selectively on the medial rectus motoneurons of the oculomotor nucleus. Their axons cross midline at the level of the abducens nucleus and course through the mediodorsal longitudinal fascicle (MLF) at a speed of about 10 µm/day. In order to examine this model as a means to characterize the response of central nervous system neurons to the injury of their axons. The sectioning of the MLF was performed by a microknife aimed through the cerebellum with an anteriorly directed angle. The axons of the abducens nucleus nerve transected in the MLF -1 mm caudal to the trochlear nucleus. Abducens internuclear neurons observed at 6, 14 and 28 days post-injury showed marked ultrastructural changes. In contrast to control unlesioned cells, the cells had a diminished and disorganized rough endoplasmic reticulum and a hypertrophic Golgi apparatus. The density of axonan synapses was noticeably reduced in the abducens internuclear neurons. Instead numerous filamentous or multilayered glial processes appeared covering large areas of the somatic membrane. Postembedding immunocytochemistry of semithin sections against GFAP indicated an increase in immunopositive profiles both in the neuropil and surrounding the somata. Biocytin-labeled axons of abducens internuclear neurons could be followed in parasagittal sections along the MLF caudal to the lesion site. Axons did not penetrate across the scarring tissue, but coursed in abaxial trajectories like U-turns and round angles. Axonal terminals formed either big and smooth clubs or sprouted into several short and thin collaterals exhibiting both terminal and en-passant bouton-like structures. In spite of the failure of axons to pass through the lesion site, the presence of these bouton-like elements in the MLF caudal to the lesion might represent some form of axonal regeneration in the central nervous system.

T07.8

Motor axons regenerating in mixed nerve preferentially reinnervate former motor pathways. However, the influence of regenerating sensory axons on this process is unknown. These experiments examine the specificity of motor axon regeneration after DRG exclusion. "Pure motor" cranial nerves were prepared in 1 month old rats by excision of the ipsilateral III, IV and VI DRG's. Three groups of 20 experimental nerves were prepared by DRG excision and simultaneous repair of the proximal femoral nerve, followed by an eviscerating a 4 mm gap within silicron tube. Nerves were evaluated at 2, 3 and 8 weeks by double labeling femoral sensory and motor branches with HRP and AChE. Labeled motor neurons were counted and scored to their projection into the motor branch (M), sensory branch (S), or both branches (double labeled) (D). Mean motor neuron counts (N = 20/gm) were: 2 weeks: M=126, S=57, DL=31; 3 weeks: M=194, S=87, DL=33; 8 weeks: M=219, S=76, DL=35. In the 8 week group, a mean of 834 myelinated axons reinnervated the motor branch and a mean of 517 reinnervated the sensory branch. Motor neurons preferentially reinnervated the motor branch at all times. In comparison with previous studies, specificity was greater and occurred more quickly when sensory axons were removed. Motor neurons retained twice as many axon collaterals when projecting to the sensory branch as opposed to the motor. 

T07.9
WITHDRAWN

T07.10

The purpose of this study is to quantify the cellular and extracellular characteristics of nerve degeneration/regeneration using volume fraction and cell density analysis of electron micrographs. Randomly transected (n=10) were selected from 7 dp injured nerves (n=6) at regions taken before, at, and after the front of regenerating axons, determined with the vibrating probe. The mean nerve fiber density dropped significantly in the distal degenerating nerve segments when compared to proximal normal nerve; the degenerating fibers were still being removed by macrophages. Conversely, the extracellular space, which is related to tissue edema, showed a significant increase when compared to normal nerve. Volume fraction values were significantly increased in the distal regions for Schwann cells, macrophages, and fibroblasts, while cell density data suggested the fibers were reduced, but did not increase in cell number. These findings are consistent with the tissue swelling and cellular proliferation characteristics of inflammation. As expected, both volume fraction and density values decreased as the degenerating axon diminished at regions more distal from the lesion site. This ultrastructural portrait of spreading axons regenerating through the terrain of degenerating nerve is consistent with current hypotheses. Finally, these comparisons of specific volume fraction and cell density values may improve our understanding of MRI studies of injured peripheral nerve. Supported by NIH grant NS19790, BSRG 50784RTF and the Enelow Fund.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
T.07.11
OCCURRENCE OF EPIDERMAL NERVE ENDBRANCHMENTS IN GLABROUS AND Hairy SKIN of the Rat FOOT AFTER SCARIFIC NERVE REGENERATION. N. Stanimirović1, T. D. Johannsen2, S. Hildebrand1,2
Dept. Cell Biology1, Dept. Plastic Surgery, Hand Surgery & Burns2, Faculty of Health Sciences, University of Linköping, Dept. Neuroscience1, Karlanska Institute, Stockholm, Sweden

The occurrence and distribution of intraneural epidermal nerve endings in the rat foot was examined in normal cases and three months after sciatic neurotomy/suture or a crush lesion. Nerve endings were visualized in electron micrographs sections with the use of antibodies against protein gene product 9.5. Normal glabrous skin exhibited 23.3 endings/mm length. Neurotomy/suture cases had 6.1 endings/mm length. Acute regeneration was normal, but the intraneural nerve endings tended to be abnormally short and occurred mainly in the basal layer of the epidermis. In sections from hairy skin countings were not possible. Subjective evidence indicated that the occurrence of dermal and epidermal axon profiles usually was deficient after neurotomy/suture and normal after crush. Skin samples from the contralateral side of operated animals showed a normal occurrence and distribution of nerve endings. Cases subjected to neurotomy/suture showed increased numbers of immunoreactive intraneural cells and an abnormally thin epidermis. A deficient regeneration of intraneural nerve endings may be one factor behind the unsatisfactory restitution of sensory function after neurotomy and suture.

T.07.13
Histochimical Analysis of Sensory and Motor Axons and Neuronal Cell Bodies Following Anoxia of the Median-Ulnar and Sciatic Nerves of Adult Rats. Macias, M.V., A. Ribeys, D.A. Lehman, C.T. Department of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53236

Current studies on peripheral nerve regeneration do not determine the regenerating fibers of either alpha motor or sensory axons. Carboxic anhydrase (CA) and cholinesterase (CHE) histochemical activities of nerve fibers distinguish angulated sensory and motor fibers, respectively. In our previous studies of the rabbit sciatic nerve, regenerating sensory axon sprouts appeared in the earlier and in greater numbers than motor axons. A rat model was developed to determine whether axonal regrowth could be dependent upon the distance from the site of injury, and if so, whether neurons are triggered to sprout following axotomy by an early degenerative signal from the injury site. The right median-ulnar and left sciatic nerves of male Sprague-Dawley rats (225-275g) were studied. Motor and sensory cell bodies are at a similar vertebral level for the median-ulnar nerve in the sciatic nerve, motor cell bodies of both nerves are by 48 mm, and the proximal stumps sampled 10, 30, or 90 days later. Histochimical staining for carboxic anhydrase and cholinesterase was performed on serial cryostat sections, and the ratio of CA/CHE positive regenerating fibers was counted. For transport studies, the nerves were crushed, injected at crush site with 5 µl of 1% WGA-HRP, ligated and automated distal to crush site. HRP activity was visualized in the dorsal root ganglia and ventral horn of the spinal cords using tetra-methyl benzidine histochimistry. 12, 24, and 48 hr. after injection, the CHE ratio of positive to negative regenerating and sciatic nerves was 2.5 to 3.25 and 1.6-2.1 in control (normal). 75.5±2.0 and 68.6±2.35 at 10 days. 60.5±2.0 and 63.1±1.6 at 30 days. and 67.5±1.6 at 90 days respectively. The transport studies showed dorsal root ganglia labelling for both nerves at 12 hrs. Motor cell bodies of the median-ulnar nerve were labelling at 12 hrs, but those of the sciatic nerves were not labelled until 48 hrs. Results show that axon regeneration is directly related to the distance between soma and axon site and are consistent with injured neurons being signaled to regenerate by a retrogradely transported factor. (Supported by NIG-022).

T.07.15
Long-term Changes of Motor Unit Organization after Peripheral Nerve Repair. A. Mocetti, M. Lehnhert, Ch. Braun and A.C. Nacimiento*, Neurosurgical Research Laboratory and Department of Trauma Surgery, Saarland University, Medical School, 66421 Homburg/Saar

Previous work from our laboratory demonstrated that end-to-end or graft repair of the albino rat sciatic nerve histochemical profile of reinnervated extensor digitorum longus (EDL) muscle fibers in the rat by inserting fiber type conversion leading to an additional fiber type (SDH-INT) with reactivity between those of type IIA and IIB fibers. This type is not present in normal EDL of young adult rats. An early, bilateral increase in number of SDH-INT 3 months after repair was followed by a decrease after 6 months, indicating partial recovery of motor unit organization pattern. To analyse degree and scope of this reversibility we extended our investigation to 15 months postoperative. After computer-assisted quantification of histochemical typing, the values obtained were compared with those of animals with intact intervention of the same age. Results: SDH-INT number increased significantly through conversion of IIA fiber type on the operated, but not on the contralateral side after end-to-end repair. No differences were observed after grafting. In intact muscle SDH-INT fiber type was also present, but to a lesser amount. Conclusion: Postrepair alteration of motor unit organization was not reversed with time, suggesting a reduction of motor unit plasticity enhanced by repair, the link between reinnervation and concomitant age related changes of fiber type composition.

T.07.12

In recent years the study of the human skin innervation new impetus by immunohistological techniques. There are, however, only a few studies dealing with functional markers in both human and animal. Previous investigations performed in our laboratory demonstrated the presence of nervous structures, identified by means of 3H-thymidine autoradiography in human nerve transplants. In the present work we analyzed the immunohistochemistry for both structural (Pgp 9.5) and functional markers of sensitive nerve fibers (calcitonin gene-related peptide (CGRP), substance P, and neurotrophin-3) in transplants up to 8 years following the surgical procedure. In the human skin transplants we observed the immunohistochemical reaction for both structural (Pgp 9.5) and functional markers of sensitive nerve fibers (calcitonin gene-related peptide (CGRP), substance P, and neurotrophin-3) in transplants ranging from 3 to 8 years. The Pgp 9.5 immunoreactive structures are qualitatively distributed in a similar pattern both in normal and transplanted skin, although a marked reduction was observed in the last. We detected the presence of Schwann cells and non-innervated Merkel cells, intraneuronal and, in a limited number, of capillaries. Immunoreactivity for CGRP is present in almost all the structures detected with Pgp 9.5 (including some of the Merkel cells, but not the capillarized receptors), although their total number is greatly decreased. Finally in some samples a very limited number of SP-immunoreactive structures (mainly intraneuronal and intradermal fibers) was observed. In our study we showed that regenerating nervous structures in human skin may exhibit immunocytochemical markers indicating a potential functional activity. Their number is greatly reduced in comparison with normal skin, but their qualitative distribution seems not altered, suggesting the regeneration involves all the different classes of nerve fibers innervating the skin. Further quantitative studies are required to understand if functional recovery of the transplanted skin innervation of functional markers might be related. This work was supported by FPPSI.

T.07.14
CELLULAR FACTORS INVOLVED IN NEUROMA FORMATION. D.M. Ziarro, R.W. Beumer, S. Zhao, H. Tran, D. Kline, H. Gould*. Deps. of Cytphology, Neurosurgery, and Neurology, LSU Medical Center, New Orleans, LA 70112

Nerve growth factor (NGF) and its low-affinity receptor (NGFR) have recently been detected in the distal segment of the sciatic nerve 6 hours to 14 days after transection. We have investigated cellular localization of NGF, growth associated protein (GAP-43), basic fibroblast growth factor (bFGF), and GFNR1 at 2 weeks post-injury in a 12 month neonatal rat model and in human neonates. In the monkey 2 weeks after transection, labile nerve proximal segment immunoactivity for NGF was found in the perineurium and less intensely in endoneurium. In both monkey and human neonates, intense staining was associated with disintegrating fibers. We also found staining of Schwann sheaths surrounding masses of axons. Immunoreactivity for NGF was more intense in 12-month monkey specimens and in human neonates than in 2-month-week neonates. Proximal segments of the neonate showed equal axonal staining for GAP-43, whereas very few thin disorganized fibers were stained with GAP-43, and the number of GAP-43-stained fibers decreased with time of nerve development. Western blot of 3-month monkey neonate showed a decrease in bFGF and GFNR1, compared with the proximal segments. In conclusion, the increase in NGF, together with the decrease in GAP-43, bFGF, and GFNR1, may be related to the formation of the neuroma. However, the role of NGF in the development of the axon mass of the neuroma is not clear. Supported in part by DAMD17-93-V-3013.

T.07.16
TYROSINE HYDROXYLASE-IMMUNOREACTIVITY IS EXPRESSED IN DORSAL ROOT GANGLIA OF CHICK EMBRYOS AFTER SPINAL CORD TRANSSECTION. P.A. Guitton A. Y. Gonzalez, K.D. Kemp and L. Wallace*. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131

Catecholaminergic profile traits, such as the expression of tyrosine hydroxylase (TH), are normally not associated with cells of the dorsal root ganglia (DRG). However, cells derived from embryonic quail DRG synthesize TH as well as catecholamines when grown in culture, although these properties are not found in normal developing embryos (SO). In contrast, in cultures of TH-immunoreactive (TH-IR) cells are present in many DRG in chick embryos, and we report here a striking change in the number of these cells that have undergone spinal cord transection. Animals underwent complete unilateral thoracic spinal cord transection either on E12 or E14 according to the method of Hasen et al. ’93 (all animals were prepared in the laboratory of Dr. J. Steeves, Univ. Of British Columbia), and were later sacrificed on E20. Analysis of embryos transected on E12 revealed an increase in TH-IR cells within DRG throughout the thoracic cord, although the increase was most prominent at the level of initial cord lesion with up to 23-30 TH-IR cells observed per 10 µm section of DRG. The increase in number of TH-IR cells decreased markedly in both cervical and lumbar cord segments. Control unlesioned animals E21 demonstrated at most 2-3 immunostained cells per section of DRG at any cord level. In embryos lesioned on E14 the TH-IR cells dispersed throughout the DRG and appeared as primary sensory neurons ranging from 20-30 µm in diameter; some with a single thick immunostained axon. In contrast, no comparable changes were discerned in embryos transected on E12. It is possible that the expression of TH by chick embryonic DRG neurons results from injury to central projections of the cells or appears as a response to synaptic rearrangements and exposure to substances in the thoracic cord during the recovery period. Supported by DHHS-AG08222 and RR08139.
FORMATION OF SYNAPTIC CONNECTIONS IN THE PTEROPOD MOLLUSC CLONE LIMACINA: REGENERATION AND CELL CULTURE STUDIES. Yu. Rashnov, L. Popov, P. Zelenski, R. Sadovyi. Belorussky Institute of Physico-Chemical Biology, Moscow State University, 119899, Moscow, Russia.

The neuronal network controlling rhythmic wing movements in the pteropod mollusc Clione limacina is located in a pair of pedal ganglia interconnected by a commissure (C). Neurotransmitters comprising the network, as well as their connections have been identified. The motoneurons project to the wing muscles via ipsilateral wing nerve (N). The interneurons provide coordination between the left and the right wing by means of their axons in C. It was shown that, in the otherwise intact animals, the original connections of the moto- and interneurons regenerated after crushing their axons. A new technique to study this regeneration in vitro was used in the present investigations. The isolated pedal ganglia were embedded in agarose gel and cultured in different positions to each other: NN - the stump of the wing nerve of the right ganglion contacted the stump of the left wing nerve; NC - the stump of the wing nerve of the ganglion contacted the stump of the commissure of the other ganglion; CC - the stump of the commissure of two ganglia contacted each other. Interneurons were able to outgrow only through the CC contact. In this case, they restored correct connections with their original targets. In two other cases the interneurons sprouted in their own ganglion only. The motoneurons were able to cross all these types of contact. They formed chemical connections which were not present in the intact ganglia, the connections resembled the neuromuscular acetylcholinergic synapses formed by the motoneurons on the muscle fibers. The abnormal chemical connections were also found in the pairs of cultured isolated identified motoneurons. (Supported by ISF grant MIU000 and KVA grant for Swed.-Russ. sci. coop.).

STAINING, TRACING, AND IMAGING TECHNIQUES III

T08.1
DIRECT AND INDIRECT DETECTION OF FLUORESCENCE LABELLED NUCLEIC ACID PROBES FOR IN SITU HYBRIDIZATION. J. Duratt* and S. Branning, Amersham Laboratories, Amersham, Buckinghamshire, HP7 0LL, UK.

In situ hybridization is a powerful technique for the study of mRNA, genomic sequences and viral infection within the structure of cells, tissues and chromosomes. A non-radioactive system has been developed which uses fluorescein as a label for DNA, RNA and oligonucleotide probes. The efficiency of the labelling reaction can be monitored in a rapid, semi-quantitative assay based on the fluorescent properties of the label. The system uses a hybridization buffer which is formulated to enhance specific signal and reduce background. Various detection procedures have been utilised to reveal the fluorescein labelled hybrids. Comparison has been made in terms of resolution, sensitivity and speed for direct and indirect detection of the fluorescein label. The direct method utilises fluorescence microscopy whilst the indirect methods utilise anti-fluorescein antibody conjugated to either alkaline phosphatase, HRP or gold and detected using appropriate substrate systems. These include NBT / BCIP, cobalt enhanced DAB, luminol based chemiluminescence or silver enhancement. Model systems used in this work including POMC detection in rat pituitary sections.

T08.2
A RIBONUCLEASE-RESISTANT AND REPRODUCIBLE METHOD OF IN SITU HYBRIDIZATION HISTOCHEMISTRY IN RAT BRAIN TISSUE. M.E. Wolf*, Y. Chen and W.W. Lu. Dept. of Neuroscience, Finch University of Health Science/ The Chicago Medical School, North Chicago, IL 60064

The major problems limiting neurological applications of in situ hybridization are: 1) contamination by ribonuclease (RNase), which is difficult to avoid and therefore makes the method difficult to establish for many laboratories, and 2) lack of reproducibility, which makes the method inadequate for detecting and quantifying changes in mRNA levels. Based on a previously described method for free-floating brain tissue (Brain Res. 578: 135, 1992), we have developed a modified method of in situ hybridization that addresses these problems. Briefly, rat brains are perfused with saline containing 0.02% diethyldithiocarbamate, a RNase inhibitor, followed by fixative solution (4% paraformaldehyde). Brains are cut frozen (40 μm) and sections stored free-floating in an ethylene glycol-based cryoprotectant solution at -20°C. Brain sections are then rinsed in 50% formamide and 4 x SSC, and hybridized with 35S-labeled oligonucleotide probes at 37°C overnight. Thus, RNase resistance is afforded by the inclusion of RNase inhibitors during steps in which mRNA is vulnerable to RNase digestion, alleviating the need to maintain RNase-free conditions during experiments. These changes result in higher levels of specific hybridization, while maintaining low background. In addition, a high level of reproducibility is obtained, both for sections obtained from the same animal and for corresponding sections obtained from different animals. This method has been characterized for preproenkephalin and glial cell line derived neurotrophic factor mRNA. Supported by USPHS Grant DA 07735.

T08.3
PERIAXONAL PLASMA MEMBRANES ARE NOVEL RNA-ASSOCIATED STRUCTURES LOCALIZED SUBJUVENTIC: THE AXOLEmma OF THE MAUTHER CELL AXON. E. Koretsky.* Dept. of Physiology, Univ. at Buffalo, Buffalo, NY 14214.

Recently, ultrastructural analysis of unstained, ultrathin sections of isolated cell axon using electron spectroscopic imaging (ESI) of RNA phosphorus, revealed random clusters of 25 nm signals typical of ribosomes, which appeared only above the phosphorous absorption edge. The clusters were distributed intermittently in the cortical zone and were not present in RNase digested axoplasm (Koering & Martin Soc. Neurosci. Abstr. Vol 20, Part 2, p. 1332, 1994). Surface inspection of isolated M-cell axoplasm after staining with JOYO-I, a selective, nucleic acid fluorescent dye, reveals periaxosomal plaque-like bodies, which vary in size, shape, intrinsic organization, and fluorescence intensity. Structural correlates are often visible in phase-contrast and DIC microscopies. Associated with regional domains of some plaques are discrete fluorescent puncta, which are distributed within a volume extending into the extracellular. All JOYO-1 fluorescent structures are sensitive to RNase. Confocal microscopy after double labeling of isolated axoplasm with rhodamine-phalloidin and JOYO-1 indicates that the plaques can be superficial to and/or integrated within the cortical actin layer. Periaxosomal plaques, which are structurally labile unless stabilized in isolated native axoplasm, may be ribosome-associated domains.

T08.4

Different viral strains have been shown to be associated with patterns of intercellular transport of Herpes simplex virus (Type I) (HSV) within the CNS. McAintyre-B strain undergoes retrograde transneuronal transport, i.e., from cell bodies to nearby axons; H129 strain undergoes anterograde transneuronal transport, i.e. from axons to nearby cell bodies. Mechanisms that might account for the different viral behaviors include preferential uptake by axons or cell bodies/dendrites; a defect in anterograde or retrograde axonal transport; and different rates of viral proliferation or release. Our goal was to determine whether the behaviors could be correlated with the abilities of the viral strains to undergo anterograde or retrograde transport or with preferential infection of domains of the plasma membrane. The corneas of BALB/c mice were inoculated with equivalent titers of McAintyre-B or H129 HSV. After 3-5 days, the mice were killed, and the trigeminal ganglia (TG) and brainstem were examined immunocytochemically for the presence of HSV antigens. Both strains were transported in the retrograde direction to TG cells and labeled equivalent numbers of TG neurons. However, the McAintyre-B infected TG displayed fewer lymphocytes or HSV-positive axons or glial cells than the H129 infected TG. Although both strains were capable of anterograde transport of virus, viral infection in the n. of the spinal tract of V in the McAintyre-B infected animals was delayed by one day. We also studied the apical and basolateral plasma membrane domains of M00C K cells as models for polarized uptake by neuronal axonal and dendritic domains. Half of the cultures were treated with EGTA to disrupt tight junctions between cells and expose the basolateral cell surface. Equal levels of virus were applied to the cultures, and the number of immunopositive foci per pta inoculated was measured. The strains were identical in their preferential infection of the basolateral surface of the MDCK cells. However, the H129 strain of HSV yielded 2.3-fold the number of foci per pta as did the McAintyre-B strain virus. The increased proliferation or viral release of H129 may lead to a more robust anterograde transport of virus following viral inoculation in the CNS.
ADENOVIRUS TRANSFER OF GREEN FLUORESCENT PROTEIN FOR LABELING PRIMARY MAMMALIAN NEURAL CELLS. K. Morishita1, L.Richards1, M.H. Sakauchi1, A.Clayton5, Inst. for Immunology, Kyoto Univ., Kyoto, Japan2, The Salk Inst., La Jolla, CA2.

The jellyfish protein, green fluorescent protein (GFP), is a bioluminescent protein shown to be effective at labeling touch receptor neurons in transformed C.elegans (Chalfie et al. '94). Due to its small coding region (0.7 Kb), GFP has the potential to be used to tag gene products (Mizuno et al. '95), or as a marker of specific gene expression in a mammalian cell line. Its expression is regulated by a 600 bp promoter, which contains cytokine responsive elements. GFP has been used extensively in mammalian cells and in worms (Chalfie et al. '94), Xenopus (Wu et al. '95) and zebrasib (Tannali et al. '95), but not in mammals. We have been modifying the GFP gene and testing the efficiency of different promoter constructs to increase the fluorescence labeling of mammalian neural cells. We placed GFP under the control of the CAG promoter, the NS-1 promoter, and the mouse CAG promoter, and found that in all 3 cell lines, the GFP labeling was bright, although concentrated in the nucleus; but in primary neural cell cultures labeling was dim. To improve the fluorescence intensity we repeated the GFP mutations created by Helm, Cobb and Tsien ('95). These mutations, in addition to the CAG promoter, produced strong fluorescence. We then modified the GFP localization, and we have also made fusion constructs of the GFP with the membrane associated region of other proteins, and a tandem GFP repeat construct. The fusion constructs produce a strongly fluorescent protein localized to the cell membrane of primary neural cultures. By coupling the modified GFP-CAG constructs with the replication-incompetent adenovirus gene transfer system, we are assessing the efficiency of this system for labeling neural cells in vivo.

CATIONIC LIPOSOME-MEDIATED EXPRESSION OF BACTERIAL BETA-GALACTOSIDASE (b-gal) IN CULTURED SPINAL NEURONAL CELLS. E. Azaz1, R.C. Benjamini2, and G.W. Gross2. 1) Clinical Laboratories, Univ. of Maryland Medical System, Baltimore MD 21201, and 2) Department of Biological Sciences, Univ. of North Texas, Denton TX 76203.
The cDNA expression vectors pCMV-b-gal (Stratagene) and pSV40-b-gal (Promega) were complexed with rhodamine-labeled cationic liposomes (made of DDAB or DOPE and DOTAP, 1:1 mole ratio) and applied to 3-8 week cultures grown from dissociated embryonic (E-14) murine spinal tissue. Cells were incubated with DNA/ liposome complexes (2:1 mol charge ratio) for 4 h after which the medium was changed and cells were post-incubated for 48 hr with no resulting overt cytotoxicity. Histochecmical staining of these cultures for b-gal revealed strong staining in some glia cells of the cultures. Co-localization of cDNA-labeled cells treated only with plasmid DNA was negative for b-gal. So far we have not seen any neurones stained for b-gal. Mechanisms of molecular delivery could be (i) endocytosis, as we have demonstrated by the localization of intact liposomes inside cells with electron microscopy, and (ii) fusion, because of the strong, uniform fluorescence seen in all neurons (and some glia) treated with rhodamine-labeled liposomes. In light of strong rhodamine staining of neurons and weaker staining of glia, we anticipate that most cells in these cultures received the vectors but that only a few glia were able to express b-gal. We conclude that cationic liposomes hold promise as a delivery system for transformation of mature mammalian neurons.

IMAGE CYTOMETRY OF B-GALACTOSIDASE REPORTER GENE EXPRESSION FUSED TO GAP-43 GENE IN RAT PC-12 CELLS. Y. Liu1 and L.E. DeBault1. Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.
The cDNA encoding the N-terminal 20 amino acid sequence of the neural specific protein GAP-43 (neuromodulin) was fused with the b-galactosidase (b-gal) gene. The fusion cDNA was subcloned into pCDNA I-neo (Invitrogen) at Hind-III and Not-I sites, under the control of a human cytosmeplast promoter. The plasmid construct was transfected into a PC-12 (a neuronal and glial cell line) cell line which gave a stable but heterogeneous population of transfected cells (containing positive cells with a single or multiple copies of the plasmid and negative untransfected cells). Enzymechemistry of the B-Gal was performed using 5-bromo-4-chloro-3-indolyl-D-b-galactopyranoside as the substrate resulting in a blue stain that was measured at 620nm in a DISCOVERY™ Flu-Or bage Image Cytometry System. The positive cells showed a range of staining intensities from a basal level assumed to represent the expression of a single copy of the plasmid to values 4 to 5 times this basal level. In a few cells the expression of the B-Gal exceeded 10 times the basal level. Further work on a suitable external standard is in progress. When properly controlled this enymechemistry procedure combined with quantitative Image Cytometry can be used to monitor gene expression at the individual cell level. (Supported in part by NIH grant NS 18776 and an OCAST to L.ED. and NSF grant IBN-9119976 to Y.L.)


We demonstrated a neural cell-type specific gene expression using adenovirus vector, which is useful for delivering foreign genes into quiescent neural cells. We produced eight recombinant replication-defective adenoviruses carrying the LacZ reporter gene driven by various promoters, including those of L7/PCP2 gene (highly restricted expression in cerebellar Purkinje cells), and the myelin basic protein (in oligodendrocytes) gene. We demonstrated that in vitro and in vivo promoter-driven, neural cell-type specific gene expression by recombinant adenovirus. Primary cultures of mouse cerebellum were infected with these adenoviruses. When we observe LacZ gene expression only in Purkinje cells with L7-LacZ vector and in oligodendrocytes with MBP-LacZ vector. We also introduced these adenovirus vectors into the organotypic slice culture of mice cerebellum and in vivo injection into rat cerebellum. These cell-type specific adenovirus vectors make it possible to study the molecular mechanism underlying, development and functional organization of the nervous system. Thus, adenovirus vectors are useful for cell-type specific therapeutic uses and studies requiring neural cell-type specific gene expression.


Thy-1 is a cell surface protein of the immunoglobulin superfamily, expressed on all neurons except those of olfactory epithelial origin. We have attempted to take advantage of the widespread neuronal expression of this gene by using it to produce a novel marker for cell lineage studies in the developing cerebral cortex. Our initial strategy was to use the Thy-1 promoter and regulatory regions to drive the expression of a bacterial marker protein. Due to the complexity and apparent positional dependence of the Thy-1 enhancer elements, we were only moderately successful (Soc. Neuro. Abpt., 19: 1260, 1993). Our next approach was to make a minor modification to the Thy-1 gene; the signal sequence was replaced with a unique 8 amino acid sequence ("FLAG"") epitope (IBL, New Haven, Conn.). In the resulting cytoplasmic epitope-tagged Thy-1 (CET) transgenic mice, CET transcript was expressed only in tissues that normally express Thy-1 and CET protein was localized to the cytoplasm (Soc. Neuro. Abpt., 20: 57, 1994). We now report the production of homzygous mice from one transgenic line. Quantitation of mRNA levels by protection assay suggests that the level of CET transgene mRNA in homzygous mice is about 12 times the level of endogenous Thy-1 mRNA. Preliminary immunocytochemistry in homzygotes reveals widespread expression of the transgene in the cortex. In addition, northern analysis indicates the developmental expression of CET mRNA parallels the expression of Thy-1 mRNA, suggesting that the transgene is regulated in the same manner as the endogenous gene. We are now in the process of identifying, by in situ hybridization, the cell types that express the CET transcript and are optimistic that we will be able to begin our cell lineage studies soon.

HINTS OF CELLULAR RESOLUTION USING RETROGRADE VOLTAGE-SENSITIVE DYE STAINING IN THE EMBRYONIC CHICK SPINAL CORD Y. Tsai1, L.B. Coleman1, and C. Hickie. Department of Physiology, Yale University School of Medicine, New Haven, CT 06520

Werner et al and Tsai et al (ISN, 1994) showed that hydrophobic, positively-charged styryl dyes would retrogradely label motoneurons in the embryonic chick spinal cord after injection into ventral roots. However, the activity-dependent signals on neighboring detectors of the photodiode array were always shown. But the course of the signals was relatively smooth. Thus, it seemed that each pixel was detecting the averaged activity from a substantial population of motoneurons. After making several changes in experimental procedure we have detected more localized spike-like signals. First, we used a brighter light source (250 vs 150 watt xenon arc bulb) and an objective with larger magnification and higher numerical aperture (63x 1.25 NA) which improves the signal-to-noise ratio. Second, we used older animals (E14-E16) with the identical phototransistor and might be less non-specific. Third, we used near-threshold stimuli to the rostral cord in an attempt to spread out the timing of motoneuron responses. With these changes we have detected spike-like signals that were localized both spatially and temporally over the photodiode array. Thus, we may be close to being able to detect the activity of individual motoneurons in the embryonic chick spinal cord. Supported by NIH grants NS08347 and NS207102.
STAINING, these employed extent (250-350 study Laval, neurons. of function be simultaneous pulses 708.13 pulses were dissolved in 0.5 M NaCl (k-acetate), for the simultaneous juxtaacellular recording and tracer iontophoresis. Low intensity positive current pulses (5-10, 200 ms on/200 ms off, 5 to 30 ma), injected through the bridge circuit, module cell firing such as to keep it alive. Survival periods of 3-24 hour (for biocytin or Neurobiotin) and of up to 5 days (for biotin dextran) yielded the best dendritic and aonal stains. Rat thalamocortical cells, for instance, which were juxtaacellularly labeled, could be traced to their most distal axonal processes in the cortex. Electrophysiological and histochemical data show that neuronal filling occurs during a cellular micro-puncture, electrically-induced axonal or dendritic membrane patch. Control experiments consisting in the selective killing of previously injected cells provide convincing evidence it is the stained units that was recorded extracellularly and "stilled" by the juxtaacellular iontophoretic pulses. This single-cell staining method labeled used to label several types of rat brain neurons, including interneurons. Its success rate (85-100%) far exceeds that obtained by direct intracellular injections of tracers as shown by the labeling of a large sample of 94 motoneurons (from 113 animals) in the spinal cord (250 nm radius).

Provide care is taken to avoid the possible drawbacks and pitfalls that are illustrated and discussed, this novel juxtaacellular staining method represents an ideal directed Golgi-like labeling tool for studying interactions between the structure and function of individual central nervous system neurons. Supported by a grant from MRC of Canada to M. Deschenes.

708.13 SINGLE-CELL LABELING BY JUXTACELLULAR APPLICATION OF ANTEROGRADE TRACERS. D. PinaultNeurobiologie, Université Laval, HULL, (401) 663-2474, Québec, G1V 0A6, Canada.

We describe a novel method with unique advantages for the Golgi-like labeling of single neurons identified and recorded extracellularly. This procedure involves the use of fine glass micro-pipettes (tip diameter about 1 mm), which contain biocytin, Neurobiotin or biotin dextran dissolved in a salt solution (1.5% in 0.5 M NaCl or K-acetate), for the simultaneous juxtaacellular recording and tracer iontophoresis. Low intensity positive current pulses (5-10, 200 ms on/200 ms off, 5 to 30 ma), injected through the bridge circuit, module cell firing such as to keep it alive. Survival periods of 3-24 hour (for biocytin or Neurobiotin) and of up to 5 days (for biotin dextran) yielded the best dendritic and aonal stains. Rat thalamocortical cells, for instance, which were juxtaacellularly labeled, could be traced to their most distal axonal processes in the cortex. Electrophysiological and histochemical data show that neuronal filling occurs during a cellular micro-puncture, electrically-induced axonal or dendritic membrane patch. Control experiments consisting in the selective killing of previously injected cells provide convincing evidence it is the stained units that was recorded extracellularly and "stilled" by the juxtaacellular iontophoretic pulses. This single-cell staining method labeled used to label several types of rat brain neurons, including interneurons. Its success rate (85-100%) far exceeds that obtained by direct intracellular injections of tracers as shown by the labeling of a large sample of 94 motoneurons (from 113 animals) in the spinal cord (250 nm radius).

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This study analyzed whether the pig paramyxovirus of the blue eye disease was retrogradely transported from peripheral nerves to the CNS. In five suckling pigs (3-8 days old) and four cats (300-2500 g) a dose of 1 ml of paramyxovirus with 256 hemagglutinating units was injected in left medial gastrocnemius (MG) muscle or intradrural in the skin of the area innervated by the left sural (SU) nerve, or, it was also inoculated per nasum. After 11 - 40 days of injection or 5 -12 days of inoculation the animals were anesthetized with sodium pentobarbital (30 mg/kg) and fixed by intracardiac perfusion with perfusion with paraformaldehyde (6%) and glutaraldehyde (3%). Several distal to proximal segments (5 mm) of left and right (control) GM, SU, sciatic (SC) and nasal (NA) nerves as well as proximal and distal segments of LG and LG ventral and dorsal roots were cut and processed for paraffin embedding. Transversal sections of spinal cord, brain cortex, hippocampus and cerebellum were also processed for embedding. The presence of the virus was detected with conventional immunohistochemical method using mouse polyclonal antibodies against the whole inactivated virus, which was reacted with rabbit anti-mouse IgG labeled with fluorescein isocyanate. In pigs, the virus was detected in distal and proximal cells (from 30 nm) in the subnuclear complex of 32 rats.

708.15 COMPARISON OF THE UPTAKE AND TRANSPORT OF SOME COMMONLY USED SUBSTANCES. D. Ling and C.N. Izac, Dept. of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St, London, NW3 2PF, U.K.

A variety of retrograde tracers have been used over the past two decades to study the projections of central and peripheral neurons. Although many of these substances are well established some of the more recent additions have not been critically examined. The purpose of the present study was to assess the properties of a number of classical and contemporary retrograde tracers and to compare their sensitivity.

Experiments were performed on anesthetized male Sprague-Dawley rats (250-350 g). Individual tracers (HRP, cholera-toxin (CT) conjugated to HRP, fluorescent latex microspheres (300nm), WGA conjugated to colloidal gold particles (1 and 5nm) and Fluoro-Gold) were pressure injected (10-15 mum) into the tongue or hypoglossal nerve. After a suitable survival period (2-7 days) rats were perfused fixed and serial coronal sections (50 mum) of the medulla (at the level of hypoglossal nuclei) were cut on a vibrating microtome. The retrogradely transported substance was visualized using the appropriate method. Following tongue injections extensive labelling of hypoglossal motoneurones was observed with CT-HRP and Fluoro-Gold and to a lesser extent with HRP. However, following the injection of either WGA-gold or latex microspheres very little retrograde labelling was observed. In contrast, injection of all the other retrograde tracers resulted in labelling of hypoglossal motoneurones.

This study demonstrates that some of the more recently reported tracers employed in neuronal retrograde tracing studies are indeed retrogradely transported by axon terminals. Thus, previously reported retrograde transport achieved with these tracers must be attributed primarily to uptake and transport by damaged fibres at the site of injection. The results from these studies and that many of the current tracers are cholera-toxin and Fluoro-Gold, both of which are taken up by axon terminals and produce more numerous and extensive labelling of neurons.
T09.1 ANTIGEN RETRIEVAL FOR PARAFFIN SECTION IMMUNOCYTOCHEMISTRY WITH ANTIBODIES COMMONLY USED IN STUDIES OF DEGENERATIVE DISEASES. J. H. Wright, Jr.* and L.A. Macie, Deps. of Psychiatry and Pathology, New England Medical Center and Tufts University School of Medicine, Boston, MA 02111
Paraffin sections are useful for immunocytochemical studies of brain tissue obtained at autopsy and fixed for several weeks has presented difficulties with sensitivity. Recent methods of pretreatment using wet heat (‘antigen retrieval’) have shown more reliable detection of many antigens at higher antibody dilutions. The most convenient and effective methods in our hands include 10 min. autoclave or 20-30 min. at 90-95°C (heating buffer pH 6.0). Backward staining and induction is reduced by these pretreatments. A streptavidin-peroxidase kit (Biogenex) and DAB or DAB plus nickel as chromogen is used. With these methods, the following antibodies, commonly used in human sections, stain in paraffin sections from autopsy brain tissue of patients with Huntington’s disease, Alzheimer’s disease or controls, fixed in room temperature buffered formalin for at least 2 weeks.
Antibody Type Antigen Source Dilution
SM-31 mono phosph. NF Sternberger-Monoclonal 1:100.000
10-D5 mono ATP-ase Action 1:10.000
MAB1510 mono ubiquitin Chemicon 1:50.000
AB748 poly collagen IV Chemicon 1:100.000
N1506 poly GFAP Dako 1:300
Supplier’s recommendations for dilution include: SM-31 1:1,000, AB748 1:320, N1506 1:1. Wet heat pretreatment for AB substitutes for formic acid pretreatment. These methods promise to allow a wider research use of routinely prepared paraffin-embedded brain tissue.

We have established high density primary cultures of neural cells from second trimester human fetal brains (J. Neurosci. Res., 1994). Here, we report that these neural cultures can be dissociated to single-cell suspensions, sorted (PS) by size using flow cytometry (low angle forward light scattering) and re-seeded to yield cultures selectively enriched for neuronal and glial populations. Neural cell analysis was initially performed on a FACSscan and cell sorting was performed using the FACSTAR Plus sorter. With our methods, two different regions (REG1 and REG2) could be simultaneously defined by electrophoretic definition on the FACSTAR Plus sorter. Sorted neurones under REG1 were highly homogeneous and viable. As early as 3 days, 95% of these neurones were viable. The cells evaluated proceeded to elaborate processes. Somatal area was ~30 μm² and lengths of the processes measured were ~ 15 μm. By 1 DIV-PS, numerous long processes were elaborated. By 3 DIV-PS, most of the small cells were process-bearing and all were glutamate acid decarboxylase (GAD) + , neurofilament protein-60 (NF-60)+ and glial fibrillary acidic protein (GFAP)-negative. It appeared that these small neurones did not proliferate. By 6 DIV-PS, analyzed somal area revealed a single component of about 50 μm² and lengths of the longest processes were ~46 μm. These cells expressed inward currents by 3-6 DIV-PS and spontaneous electrical activity after 10 DIV-PS. In parallel, larger cells with somal area > 100 μm were recovered under REG 2 (FACS chart). These cells were GFAP+ , NF-60 negative and were not excitable. Homogeneous cell populations form human brain are particularly interesting for the evaluation of neural dysfunctions that can not be studied in animal models.

Two techniques have been developed which allow for the rapid and sensitive localization of either neuronal or myelin degeneration following insults by a variety of neurotoxins. Different classes of neurotoxins used included organophosphate, iodidomycin or kainic acid, oxidative free-radical generators (iron or manganese salts), inhibitors of mitochondrial respiration (3-nitropropionic acid), and pyridoxal phosphate/GABA depleting agents (asianic acid); Neuronal degeneration was demonstrated by the use of fluorescent dyes which labeled the cell bodies and proximal dendrites of degenerating neurons. This labeling pattern was generally comparable to that seen using conventional methods-I.e., silver-staining or immunoperoxidase methods. To facilitate multiple labeling studies, green (Fluoro-Jade) and red (Fluoro-Ruby II) fluorochromes were developed. These probes can be combined with fluorescent antibodies, apotosisk markers, axonally transported tracers, or costainers of complimentary emission color to simultaneously reveal the degenerating neurons with the appropriate fluorochrome, chromosome fragmentation, or the connectivity of identified neurons. Degeneration of the myelin sheath was demonstrated by incubating the tissue in a heated, buffered, gold chloride solution. Depending on the nature and duration of toxicant exposure, myelin degeneration could be characterized by a swelling or fragmentation of the myelin sheath, the presence of myelin containing macrophages, or the absence of normal myelin staining. The aforementioned two methods provide a simple, reliable, rapid and sensitive means for detection of neuronal or myelin degeneration following exposure to different classes of neurotoxins.

T09.4 CONVENTIONAL AND FIELD EMISSION HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY OF CEREBELLAR SYNAPSES. G.J. Castiglioni*, Instituto de Investigaciones Biologicas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela.
Telost fishes, prime and human cerebella were processed for conventional (CSEM) and field emission high resolution scanning electron microscopy (FSEM) to study the outer axo-somatic and axo-synaptic faces of axodendritic, glomerular and axosynaptic synapses. Axodendritic junctions of climbing fibers and Golgi axonal ramifications were studied in gold-palladium and chromium coated samples. CSEM of cryofractured glomerular synapses exhibited the outer axo-somatic view "en passant" mossy fiber glomeruli and the inner view of transversally and sagittally fractured glomeruli. The cryofracture method exposed the axosomatic contacts of basket axonal collaterals upon the Purkinje cell and the climbing fiber bulbous endings upon the Golgi cell. FSEM of parallel fiber-Purkinje fiber spines showed the three-dimensional structure of the synaptic membrane complex. The spheroidal synaptic vesicles appeared embedded in a homogenous axoplasmic substance. Round subunits, 15-20 nm in diameter, were observed at the postsynaptic membrane and the postsynaptic density, presumably related to neurotransmitter receptors.

Quantitative structural information on synaptic components, including synapses, synaptic structures, and dimensions of the presynaptic dense bodies of 'active zones', is commonly obtained from ultra-thin sections visualized with transmission electron microscopy. Problems arise from loss of structural information resulting from projection of a section of a three-dimensional structure onto a two-dimensional photographic plate is necessary. The relation between the true size distribution and the theoretical and actually observed size distributions of projected images is discussed for the case of a population of spherical synaptic vesicles. The observed distribution is demonstrably degraded due to the non-observation of optically thin sections of small radius. Consideration of the degradation of images is used to devise a method for interpreting the observed distribution in terms of the true distribution. This approach provides a more rational measure of the population mean and coefficient of variation. For other, non-spherical, synaptic structures (dense bodies and synaptic area), available information on the uncertainties of the observations is used to determine methods for best estimates of the true structural dimensions. (Supported by Medical Research Council, National Sciences and Engineering Council, Canada.)

T09.6 HOMOLOGUE OF DROSOPHILA NEURAL PROTEIN FREQUIN SELECTIVELY EXPRESSED IN CRUSTACEAN PHASIC MOTOR TERMINALS. H. I. Atwood*, M. Murphy*, L. Lindstrom*, and D. Pangl*, Department of Physiology, University of Toronto, Toronto, Ontario, CA MS5 1A8, and "Zentrum für Molekulare Neurobiologie, Institut für Neurale Signalverarbeitung, D-82426 Hamburg 20, FRG.
Two types of nerve terminal with markedly differing release characteristics, classified as phasic and tonic, have been described in crustacean motor axons. Each of these motor axons have initial high quanta output, but show less facilitation and more depression than tonic terminals (Atwood and Wcislo, 1986, Ir. Rev. Neurobiol. 28, 275-362). Individual crayfish muscles may be innervated solely by phasic or tonic motor axons, or by both types. Advantage was taken of this to study the extracellular space distribution of a specific protein factor (described by; Br J, which has been implicated in modulation of synaptic facilitation (Pongs et al., 1993, Neuron 11, 15-20). Affinity purified monoclonal polyclonal anti-fragulin antibody (1:50), raised against (Drosophila, was used in conjunction with FITC conjugated secondary antibody (1:200). Terminals were visualized with a Bio-Rad scanning confocal laser microscopy and a Nikon-40X water immersion lens. For comparison, preparations were stained with the vital dye 4-Di-2-Ap, which strongly labels both types of terminal. In all preparations tested, antibody reaction was strongest in phasic terminals, and absent or much weaker in tonic ones. This indicated that the factor was involved in the spherical axosomatic terminal, and preterminal axon branches showed faint reaction. Our results indicate that frequent, or a closely related protein, is enriched in crayfish phasic nerve endings that have high initial quanta output. (Supported by MRC Canada and Faculty of Medicine, Univ. of Toronto)
STAINING, TRACING, AND IMAGING TECHNIQUES IV

709.7 COMPARISON OF THE FM1-43 AND SV2 STAINING PATTERNS OF AMPHIBIAN NEUROMUSCULAR JUNCTIONS. A. W. Everett, S. J. Packard and J. Beattie*. Dept of Physiology, University of Western Australia, Nedlands 6907, Australia.

The styli dye FM1-43 labels nerve terminals in an activity dependent manner, presumably by becoming trapped in recycled synaptic vesicle membranes at transmitter release sites in the terminal. We therefore compared the distribution of FM1-43 labelled terminals with those labelled with a antibody to the SV2 antigen of synaptic vesicle membranes. Toads (Bufo marinus) were sacrificed by pithing and the iliofibularis was pinned-out in vitro. FM1-43 at 2 pM in Ringer containing; 2 mg/ml of anti-SV2 antibody to the SV2 antigen was added to the muscle for 5 minutes. The muscle was then washed briefly and fixed. Other muscles were fixed for staining with the SV2 antibody. Terminals on teased muscle fibres were imaged using fluorescence confocal microscopy. FM1-43 staining was demonstrated in isolated nerve terminals and adjacent nerve fibres. FM1-43 staining appeared as spots or more often as bands along the length of terminal branches at a frequency of about 2 cm/mm; some larger spots probably consisted of several bands that could not be clearly resolved. SV2 staining was similarly punctate with spots occurring at a frequency of not less than 40 cm/mm of terminal branch; individual spots were generally larger than FM1-43 stained spots and less clearly defined. Both staining procedures revealed very large differences in the intensity of staining of spots within single branches. The results support the notion that FM1-43 labels synaptic vesicles concentrated at release sites in a nerve terminal and that variation in the uptake of the dye at these sites may reflect differences in the number of vesicles or the composition of their membranes.


We used a novel three-color fluorescence immunocytological technique to simultaneously visualize innervating axons and nerve terminals, motor endplates, and muscle fiber type in the rat diaphragm. Nerve terminals and axons were labelled with an antibody to protein gene product 9.5 (PGP), endplates with a-bungarotoxin, and muscle fibers with an antibody to fast (type I) skeletal myosin. Fluorescein (nerve terminal), tetramethylrhodamine (endplate) and Cy-5 (type II muscle fibers) were used as fluorescent dyes to distinguish the three structures. The triple-labelled neuromuscular junctions (NMJs) were optically sectioned using a confocal microscope, and 3D views of the NMJs were created. Both 2D and 3D morphological parameters such as axonal diameter, terminal and endplate planar and surface areas, endplate gutter depth, and extent of overlap of terminal and endplate, were extracted from these images. The results indicate that I) in general, larger axons innervate larger muscle fibers; 2) when normalized for fiber diameter, planar and surface areas of both terminals and endplates are larger on type I fibers; 3) NMJs on type II fibers generally appear more complex; and 4) the extent of overlap is greater in type I fibers. These morphological differences may underlie fiber type differences in neuromuscular transmission. Supported by NIH grants HL34817 and HL37680.


The most potent toxins known are produced by strains of Clostridium botulinum. To paralyze the vertebrate neuromuscular junction the toxin is released into the nerve endings, is translocated into the terminal, and after activation of its enzymatic activity, hydrolyzes proteins of the exocytotic apparatus. Our goal was to develop a convenient, reliable technique to detect specific binding of Bot A to its targets. Our technique utilized fluorescently labeled latex spheres, which are capable of detecting a single receptor.

Non-specific binding sites on 7 µm thick sections of unfixed, cryostat tissue were first blocked with 20% goat serum in PBS (0.5% P/S). We incubated the diaphragm for 1 h at RT with various concentrations of Bot A in 0.5% P/S, followed by incubation with rabbit anti-Bot A antibody, biotin labeled goat anti-rabbit antibody, and finally avidin-labeled, 0.03 µm diameter, fluorescent latex spheres. As expected, binding was limited to the presynaptic membrane. The technique allowed us to visualize with light microscopy a minimum of 6 Bot A binding sites per µm² of nerve ending surface. In addition, we could detect binding on diaphragms that were exposed as little as 10 pM Bot A, which is in the low range of effective in vitro doses that block twitch tension.

This a convenient technique for detecting Bot A receptors and may be applicable to other receptors as well.


Evaluation of nerves in skin biopsy yields valuable information about peripheral nerve endings. Comparison of nerves in skin from normal vs. diabetic donors reveals striking differences in the pattern of innervation as well as the amount of nerve present in various structures of the skin.

Biopsies obtained from normal and diabetic donors (with informed consent), were fixed, sectioned and processed for double-stain immunohistochemistry using antibodies to PGP 9.5 to localize nerves and type IV collagen to localize basal membrane. Digitalized image files were obtained using a laser scanning confocal microscope.

Lengths and volume of nerve as a function of the volume of epidermis or sweat gland were determined by computer analysis and quantification of three dimensional reconstructions.

Nerves in skin can be clearly visualized using antibody to PGP 9.5. The relationship of nerve to other structures can be correlated by double staining with type IV collagen. The amount of nerve was greatly reduced or absent in the biopsies from diabetic donors as compared with controls. A similar decrease in numbers was seen in the sudomotor nerves of sweat glands in biopsies of diabetic subjects.

The data clearly indicate that skin biopsy evaluation is useful in the clinical assessment of neuropathy. Staging of neuropathies from skin biopsy data will be useful in assessing the effects of treatments such as drug therapy and pancreas transplantation.

GENE STRUCTURE AND FUNCTION V


Induction of neurite outgrowth in PC12 cells is transcription-dependent and is associated with the accumulation of tau proteins. The increase in tau proteins levels, results from an increase of tau mRNA levels which reflects increased transcription and/or stability of the message. The latter possibility was tested in PGP-123 transfected cells. We found that the half life of tau mRNA is elevated to 18 hours in induced cells as compared to 7 hours in uninduced cells.To identify tau sequences involved in mRNA stabilization, selected fragments of the 3'UTR were subcloned into the 3'end of the coding region of human c-fos, used as a reporter gene. The transfected cell lines overexpressing c-fos constructs did not show any morphological differences following NGF induction. Stable PC12 line containing construct B (about 1400 nucleotides of the 3UTR), show a significant increase of 6 folds in the steady state c-fos mRNA levels following NGF induction. The results demonstrated that the half-life of fos-B mRNA increases to 2.1 hrs as compared with 1 hr in cells transfected with c-fos alone. The half-life of fos-B mRNA is even longer when transfected cells were treated with NGF-56.8 hrs.

The effect is neuronal specific, as it was not observed in 3T3 cells transfected with the same constructs. By using a uv-cross linking assay a specific binding of proteins prepared from neuronal cells was observed while no binding activity was detected when extracts were prepared from 3T3 cells. Thus we conclude that tau mRNA stabilization is mediated by cis-signal located in the 3'UTR of the message in conjunction with trans-acting neuronal proteins.

Supported by the Israel Academy of Sciences and Humanities, the GIF Foundation and the Forshheimerin Center for Molecular Genetics (WIS).


The lack of immortalized serotonergic cell lines has been a major obstacle to the study of the tissue-specific differential regulation of tryptophan hydroxylase (TPH) gene expression in vivo and during the circadian rhythm. TPH is the first step enzyme in serotonin and melatonin biosynthesis in neuroendocrine cells of the pineal gland. Previously, we demonstrated that a 6.1 kb 5' upstream region of the mouse TPH gene directs the restricted expression of a reporter gene fused to the pineal gland and the median and dorsal raphe nuclei in transgenic mice. Therefore, to develop TPH-expressing pineal cell lines we first established transgenic mice carrying a construct consisting of a 6.1 kb of 5' flanking region fused to the SV40 T-antigen. These animals developed highly invasive pineal tumors and died at 12 to 15 weeks of age. The pineal tumors obtained from the transgenic mice were utilized to establish the immortalized pinealocyte-derived cell lines PGT-811 and PGT-812, which exhibit characteristic properties of the pinealocyte, such as TPH and N-Acetyltransferase activities. Using PGT-812 cell transient transfection analysis revealed cAMP responsive, tissue-specific enhancing and repressing elements in the 5' upstream region of the mouse TPH gene.
DNA Mismatch Repair and DNA Methylation in the Adult Rat Brain P.J. Brooks*, K. Chen, K. Chen, and D. Goldman. Sect. on Mol Neurobiology, Laboratory of Neurogenetics, NIA/NIH.

DNA repair is essential to maintaining the integrity of the nucleotide sequence of DNA. However, much data has accumulated recently on the mechanisms of DNA repair, little is known about the DNA repair capacity of cells in the adult brain. Such knowledge is important in view of the neurological deficits seen in individuals with DNA repair diseases such as Cockayne's Syndrome (CS) and DNA repair. In the present study, we have investigated the capacity of nuclear extracts from adult rodent brain neurons to carry out DNA mismatch repair. We focused on the repair of G:T mismatched base-pairs, which arise from DNA deamination or mismatch repair. Deamination of 5-methylcytosine in DNA results in the formation of a T:G mismatch. This is repaired by the AID system, which is not normally involved in DNA repair. The results of these studies suggest that neurons in the adult mammalian brain have the capacity to carry out specific types of DNA mismatch repair.

We previously reported (Nisch. Abst. 20, 100.6) that the adult brain contains high levels of DNA methyltransferase. We propose that one function of DNA methyltransferase in the adult brain is to remethylate newly incorporated cytosine residues from G:T mismatch repair following deamination of 5-methylcytosine, thereby maintaining the original pattern of DNA methylation.


Monoamine oxidase (MAO) A and B are flavoenzymes that catalyze the oxidative deamination of biogenic and xenobiotic amines. The MAO isoforms are defined by their substrate and inhibitor selectivity. MAO-A preferentially oxidizes serotonin (5-HT), whereas MAO-B preferentially oxidizes phenylethylamine (PHE). To search for domains that confer substrate and inhibitor selectivity to these proteins, we have constructed and expressed in yeast. Replacement of a MAO-A segment (residues 161-375) with the corresponding region of MAO-B, an MAO-B, and converted typical MAO-A catalytic properties to MAO-B like ones. Similar to wild-type MAO- B, AR152L555A oxidizes PHE (Vmax 325 nmol/min/mg protein, Km 354 umM) but not 5-HT. The IC50 value for deprenyl is 20 fold less than the IC50 for clorgyline. However, the reciprocal changes in which a MAO-B segment was replaced by the corresponding region of MAO-A, termed BA152-L555G, lacked catalytic activity. The lack of catalytic activity was not due to aberrant expression but rather an inactive protein as demonstrated by Western blot analysis. These results demonstrate that the MAO-A amino acids 152-366 contains a domain(s) that confers substrate and inhibitor selectivity. (Supported by NIH grants R01 MH59035 (MERIT Award), K01 NS00776 (Research Scientist Award), R01 MH57020 and Wiel Professorship).

PARTIAL CHARACTERIZATION OF A BRAIN SPECIFIC sDNA-BINDING PROTEIN. Raghunathan A, Parra-Varela T, Malhotra C, Kamakar AR. School of Life Sciences, University of Hyderabad, Hyderabad 500134, India, Dept. of Biology, Wesleyan University, Middletown, CT 06459, USA.

Tissue specific gene expression involves an interplay of DNA-protein interactions and DNA binding proteins play a pivotal role, in terms of regulation. This study involved the purification and characterization of a single strand DNA binding protein which is possibly involved in the developmental regulation of rat brain. The protein was purified using DNA-cellulose. Chromatography and DNA binding properties were confirmed using filter binding and mobility-shift assays. The protein is a molecular weight of 30-40kDa with a pI of 5.2 and is denoted as 56 sSBP. Intrinsic fluorescence spectral suggest the presence of tryptophan in a buried condition and the amino acid analysis shows an abundance of glycine and serine residues. 56 sSBP has no significant influence on conformation and melting profiles of calf thymus DNA. It showed no nucleolytic, or DNA polymerase activity suggesting that the protein may not be involved in DNA replication, repair and/or structural organization, leaving scope to its transcriptional regulation. Immunological studies on Western blots indicated its distribution specific to the brain. (Supported by a SRF grant to AR from ICMR, India, and UGC-Research scientist-B grant from UGC, India, to MCV).
11.9

AAV-VECTOR MEDIATED HUMAN HSP702 GENE TRANSFER INTO CULTURED CELLS: L. Lipinska*, F. Welsh, M. Kaplitt, W. O’Connor, M. Dang, C. Mohla, M. O’Connor, A. Freese, Div Neurosci, Univ PA, Phila, PA 19104; Graduate Hosp, Phila, PA 19146; Lab Neurosci, Rockefeller Univ, New York, NY 10011.

Heat shock protein 72 (hsp72) expression is associated with increased neuronal survival after a variety of stresses. A causal relationship between this expression and protection has not been demonstrated. Introduction into the CNS of the gene encoding hsp72 resulting in expression may permit the assessment of such a relationship.

We have developed an adenovirus associated viral (AAV) vector with the human hsp72 gene under control of a cytomegalovirus promoter (AAV- hsp72). While injected into rat cortical or striatal mixed neuronal-glia cultures, the vector directed both short- and long-term hsp72 expression. At three days, cultures were exposed to AAV-hsp72, AAV-lacZ (a control vector containing the lacZ marker gene), or PBS. Hsp72 expression was qualitatively measured after two or seven days of viral vector incubation. Using a monoclonal antibody against human hsp72, immunocytochemistry was performed. Only those cultures transfected with AAV- hsp72 demonstrated staining for hsp72, while staining for β-galacto- sidase was observed in cultures transfected with AAV-lacZ, but not in cultures transfected with AAV-hsp72 or PBS. Western blot analysis showed similar results, with inducible hsp72 protein only present in those cultures transfected with AAV-hsp72. Absence of cytosotoxicity was confirmed by a number of techniques. These results demonstrate that this vector may be used to introduce the human hsp72 gene into CNS neurons with subsequent transgene expression. We are evaluating the neuroprotective effect of this vector and implications for therapy in stroke and trauma.

11.10


The ability to transfer genes into neurons has important utility in the study of neuronal physiology and gene regulation, and for gene therapy in the treatment of diseases of the nervous system. Replication-defective, recombinant adenosine vectors have been used to infect neurons without evidence of pathogenicity. Development of virus vectors to provide cell-type-specific promoter regulation is important for many applications. We have constructed replication-defective recombinant adenosine vectors to investigate the regulation of several promoters using the reporter lacZ gene to express β-galactosidase. Initial studies have utilized rat dorsal root ganglia neurons in tissue culture to examine expression from several promoters. An adenosine vector containing the cytomeglovirus immediate early gene promoter resulted in expression of β-galactosidase in greater than 90% of the neurons 24 hr postinfection, with continued β-galactosidase expression for 30 days without evidence of cytoxicity. Infection with an adenosine vector containing the adenovirus early E1A promoter driving lacZ resulted in β-galactosidase expression in neurons. Use of the E1A promoter provides an expression system that is not active in the cell line required to package the adenosine vector, thus allowing construction of vectors containing toxic genes.

11.11

EXPRESSION OF LACE GENE USING A HUMAN SIMPLIFIED VIRAL VECTOR RECOMBINANT INTO HIPPOCAMPAL GRANULE CELLS IN VIVO. P.A. Cresap, B.L. Harm and A. Mountbatten, Creag Neuroscience Laboratory, Rockefeller Univ, Bldg 14A, Medical School and Sloan Hospital, Belmont, MA 02178.

The herpes simplex virus (HSV) gD protein is a virus component that introduces foreign genes into postmitotic neurons. The HSV-1 mutant is replication incompetent not entering the lytic cycle (THM, 1980; 14:429I), spreads minimally through brain parenchyma, and is taken up by terminals in the region of the injected site. We determined the extent to which the IE 4/5 promoter of HSV-1 would activate total gene when introduced into hippocampal granule cells. Injectates targeted for mossy fiber terminals in the dentate hilus were guided by a laminar profile of the dentate gyrus constructed from pictures to perform proper injections. The population of the cells expressed by stimulation of the perforant path were recorded by HSV-1-lacZ and immunohistochemically guided to the dentate hilus. Ejection volumes of 0.1-1µL were delivered by microdrive monitored by the injection of the solution microdispensed in the microdrive. HSV-d2Z injected into the hilus was postoperatively transplanted to granule cell bodies. We observed robust expression of β-galactosidase in granule cell bodies both on the dorsal and ventral sea 24-72h after a single injection. Dendrite trees of granule cells were filled with β-galactosidase, indicating that fiber soma could be traced to their terminal zone in stratum lucidum. Arrays of mossy fibers showed the many fiber rosettes of mossy fiber terminals. Glial cells in the hilus did not translate lacZ. HSV-1 is a highly effective vector for introducing foreign genes into hippocampal granule cells. The electrophysiological guidance can restrict expression to a particular cell type. Supported by NIN grant HD32510. R.L.N. NIH Post-Doctoral grant to P.A.C. and HD24236 grant to R.L.N.

11.12


Recombinant vaccinia virus has been successfully used to over-express genes of interest in several mammalian cell lines and in rat hippocampal slices. Here we describe a method to over-express proteins of interest in cultured rat hippocampal neurons using the vaccinia virus system. Neurons cultured separately, as described by Banker, did not express the recombinant gene product following vaccinia virus infection. In contrast, co-cultures of hippocampal neurons and astrocytes survive and express beta-galactosidase protein following infection with recombinant vaccinia virus. The percentage of infected neurons depends upon the density of the astrocyte co-cultured along with neurons. Recombinant vaccinia virus lacking the CMV promoter has been used to infect cultured hippocampal neurons. We discuss the localization of CMV II in the neurons following infection.

11.13

EXPRESSION OF HEAT SHOCK PROTEINS (HSP70 AND HSC70) AND NUCLEAR TRANSLATION INTO THE RABBIT BRAIN FOLLOWING HYPERTERMIC OR NEUROLOGICAL INJURY. P. Murnaghan and R. Brown, Dept of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ont, Canada, M1C 1A4.

The heat shock response is characterized by i) an overall decrease in ongoing mRNA and protein synthesis, ii) an induction of heat shock proteins (hsp) and hsc proteins, iii) nuclear translocation of hsp and iv) collapse of the cytoskeleton in some cell types. We have carried out Western blot and immunocytochemical studies using antibodies which are specific for recognizing constitutive hsc70 protein or stress-inducible hsp70 protein. Large neurons, such as Purkinje neurons and motor neurons of the spinal cord, express high levels of constitutive hsc70 protein, whereas their induction of hsc70 protein in response to hyperthermia is attenuated compared to the rapid and robust induction which is seen in adjacent glial cells. In the present study we have investigated whether neuronal cell populations in the rabbit brain (neocortex and hippocampus) exhibit other features of the heat shock response. Following hyperthermia a transient induction of hsp70 protein was observed primary in non-neuronal cell populations in the rabbit brain (neocortex and hippocampus). At 1 hr post hyperthermia, a redistribution of both hsp70 and hsc70 protein from the cytoplasm into the nucleus occurs in ependymal cells of the choroid plexus with a return to the cytoplasm by 5 h. Glial cells were not stressed by 5 h. Glial cells of stress-inducible hsp70 followed by a later appearance of the protein in cellular processes. Nuclear translation of hsc70 protein was not observed in several neuronal cell populations in response to hyperthermia (Purkinje, hippocampal and thalamic neurons), however it was observed in motor neurons of the spinal cord. These neurons showed a translocation of hsc70 protein into the cytoplasm followed by a return to the cytoplasm, with a temporal profile similar to that observed for ependymal cells.

11.14

CELLULAR LOCALIZATION OF HEAT SHOCK mRNA (HSC70 AND HSP70) IN THE RABBIT BRAIN. J.A. Foster* and J.L. Brown, Dept. of Zoology, Univ. of Toronto, Scarborough Campus, West Hill, Ont, Canada, M1C 1A4.

The hsp70 multicogene family is comprised of constitutive and heat- shock-inducible members. Using radioactive in situ hybridization we have shown high constitutive expression of hsc70 mRNA in certain neurons. Here we employ non-radioactive in situ hybridization to examine cellular distribution of heat shock mRNAs. At high resolution, we observed hsc70 mRNA in the cell bodies of neurons in cortical neurons layer 2 and 3, deep cerebellar neurons and brain stem neurons (BPN), we also detected hsc70 mRNA in spiral denticles processes. This indicates a denticles expression of hsc70 mRNA was not detected in hippocampal, some cortical neurons, and most cerebellar neurons. Following heat shock, neurons with the highest levels of constitutive hsc70 mRNA, i.e. BPN and DCN, showed more dense transport of hsc70 mRNA in dendritic processes. By radioactive and non-radioactive methods we do not detect hsc70 mRNA in glial cells. We have shown a strong glial induction of hsc70 mRNA after hyperthermia. For example, oligodendrocytes showed intense signal in cytoplasmic cap regions following 1 hour of hyperthermia. Later time points following heat shock showed this distribution to be more spindelike-like but the extent of mRNA in oligodendrocytes process was not comparable to that observed for hsc70 mRNA in dendritic processes of BS and DCN. We detected basal levels of hsc70 mRNA in control neurons, particularly when tissue was fixed after freezing; this signal was localized to cell bodies. Following heat shock, an immediate glial induction and a delayed accumulation of hsc70 mRNA in cell bodies of neurons was observed. These data demonstrate that neurons with high constitutive levels of hsc70 mRNA increased dendritic transport of this message following hyperthermia, while the distribution of hsc70 mRNA was localized to cell bodies.
HETEROGENEITY OF TRANSCRIPTS ENCODING ISCHIA-INDUCED HSP70 IN BRAINS OF INDIVIDUAL WISTAR RATS. Y. Yaida, W. Valentine, K.-S. Kim, W. A. Pulsinelli and T. S. Nowak
Il Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Phosphoreothioate oligonucleotides are more stable than normal phosphodiester oligos in brain, and should therefore be better antisense reagents to modify the postischemic stress response in brain. However, we have had limited success in reducing expression of the inducible 70 kDa stress protein, hsp-70, perhaps in part due to heterogeneity of the transcripts encoding this subset of the hsp70 protein family. We have therefore employed a S' amplification (RACE) method to sequence potential antisense targets in ischemia-induced hsp70 mRNAs. Male Wistar rats were subjected to 10 min 4-vehicle occlusion ischemia. After 3 h brains were removed for mRNA preparation or in situ hybridization. The primer for cDNA synthesis consisted of a 30mer previously shown to detect inducible hsp70 mRNAs, which was then ligated to a 23mer from the rat somatostatin promoter, amplified, cloned and sequenced. We obtained 5 unique sequences from this region of the SD sequence detected an induced mRNA in 30% of Wistar rats, suggesting that the sequences, rather than reflecting strain variation, may be differentially expressed in individual animals. Ongoing studies continue to characterize rat hsp70 mRNAs with the aim of developing suitable antisense reagents. (1) Lisowski et al., Biochim. Biophys. Acta 1219:94 (1994) (2) Longo et al., J. Neurosci. Res. 36:325, 1993 (3) Mesri et al., Biochim. J. 298:561, 1994

MOLECULAR INTERACTION OF NMDA AND DOPAMINE D2L RECEPTORS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS. V. D. Nair, H. B. Nimni and R. K. Mishra. Dept of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3S5; and Laboratory of Molecular Neurobiology, The Clark Institute of Psychiatry, Toronto, Ontario, Canada.

The N-methyl-D-aspartate (NMDA) receptor, a subtype of glutamate receptor, plays a key role in synaptic plasticity in the central nervous system and dopamine neurons are involved in regulating motor and cognitive behaviours. In the moderate-sized spiny neurons of the neostriatum, dopamine modulates neuronal responses mediated by activation of excitatory amino acid receptors. A deficiency of glutamatergic neurotransmission resulting in a disturbed balance between glutamatergic and dopaminergic systems within the neostriatum may play a key role in the pathophysiology of schizophrenia. However, the molecular mechanisms underlying this interaction are poorly understood. Using a human neuroblastoma cell line, SH-SY5Y cells, expressing cloned human dopamine D2L (D2L) receptor, we now demonstrate that transient expression of D2L mRNA can be regulated by the inactivation of NMDA receptors. Protein tyrosine kinase inhibitor enhanced the D2L receptor gene activation, whereas inhibition of protein phosphatase 1 or 2A decreased receptor gene induction by NMDA blockade. These results suggest the possibility interaction between NMDA and dopamine D2L receptor. Improved therapy may be obtained by developing drugs that target the molecules which regulate both NMDA and/or dopamine receptors. Supported by MRC, Canada.

EXPRESSION OF ZIF268 IN THE TEMPORAL CORTEX OF MONKEY RELATED TO LEARNING OF VISUAL STIMULUS-STIMULUS ASSOCIATION. HOKONDO* T. KANOU, T. TAKAYAMA and Y. MIYAJI. Dept Physiology, Sch. Medicine, Univ. Tokyo, Tokyo, Tokyo 113, Japan.

To investigate the molecular basis of cognitive memory in the primate, we examined expression of immediate early gene in the temporal cortex of monkeys during visual training. Three Macaque monkeys were trained to perform a pair-association task (PA) that required them to memorize the visual stimulus-stimulus association between computer-generated pictures. Another three monkeys were trained to perform a visual discrimination task (VD) which required them to memorize the stimulus-reward association by using the same set of stimulus pictures as that for the PA task. After training with 12 pairs of stimulus pictures, both PA and VD monkeys were trained using a new set of pictures. Before the monkeys’ performance reached a plateau phase (PA, 8-10th session with the new set; VD, 5-6th session), they were perfused immediately after the completion of that training session. Serial coronal sections (50 mm intervals) of the temporal cortex were stained immunohistochemically. The specificity of the immunoreactivity of each antibody was confirmed by western blotting and by antigen- absorption experiments. We found that Zif268, a transcription factor encoded by an immediate early gene, was expressed in a different manner during PA and VD learning. Zif268-immunopositive neurons were found to be distributed as patches in the inferior temporal cortex in PA monkeys that had not VD training. The patches included Zif268-immunopositive neurons in the superficial and deep layers as well as in layer IV. Quantification of the immunoreactivity revealed that the Zif268 expression levels in PA monkeys were higher in the VD monkeys in area 37 and 5T. These results suggest that the PA learning activates different gene cascades in the inferior temporal cortex, particularly in area 36, compared with VD learning, and that Zif268 may play a role in the formation of the visual associative long-term memory in the primate.


Inducible heat shock protein 70 (HSP-70) is expressed in certain regions of the rat brain following hyperthermia, ischemia and kainic acid-induced seizures. These conditions are also known to cause inhibition of protein synthesis. Here we examined induction of hsp-70 mRNA with in situ hybridization using a 32p-labelled oligonucleotide probe, and cerebral protein synthesis following in vivo i.v. administration of a 32p-labelled neutral amino acid. For each rat, hsp-70 mRNA induction and incorporation of labelled amino acid into proteins were studied in consecutive brain sections. Focal ischemia, hyperthermia and kainic acid caused selective hsp-70 mRNA expression in those brain regions showing decreases in labelled amino acid incorporation into proteins. The level of hsp-70 mRNA expression correlated with the percentage of decrease in labelled amino acid incorporation into proteins in relation to controls. Neither cycloheximide nor anisomycin induced any detectable hsp-70 mRNA expression, despite producing marked protein synthesis inhibition. These observations suggest that hsp-70 mRNA induction and protein synthesis inhibition are two simultaneous events of the cerebral stress response following a variety of insults.

Supported by CICYT (SAF94-0076) and FIS (90/131, Spain).


Klein et al. (Biochem. Biophys. Res. Commun. 205, 410-416 (1994)) reported finding two different cDNAs which encoded the precursors of the pigmented dispersing hormones, PDH I and PDH II, in the blue crab Callinectes sapidus. In the present studies, these cDNAs have been used to design specific cDNA probes which hybridized selectively with the PDH I- and II-precursor encoding mRNAs. Northern blot experiments showed that PDH I and II were expressed in eyestalk ganglia; no hybridization products were observed using heart, muscle or hepatopancreas preparations. In situ hybridization experiments showed expression patterns for PDH I and II in the eyestalk of a single animal. Staining of adjacent sections revealed that both PDH isoforms are probably expressed in different cells in the eyestalk. These results suggest different physiological functions of both peptides, as also indicated by the results of assays for melanophore activity in fiddler crabs in which PDH II displayed a 400-fold less potency relative to PDH I.

EXPRESSION OF CYTOSKELETAL mRNAs IS ALTERED AFTER AXOTOMY IN MATURE AND AGED F344 RATS. J.M. Jacob*, B. Srinivasan and A.B. Whittemore. Dept. of Anatomical Sciences, OHUASC, OKC, OK 73190.

Axotomy results in a series of morphologic, biochemical and physiological changes in neuronal cell body. Axotomy also alters structural protein mRNA expression. In this study, the expression of several isoforms of β-tubulin (classes I, II, and IV) as well as the neurofilament triplet protein (NF-L, NF-M, NF-H) was examined in mature (24 mo) and aged (24 mo) F344 rats after axotomy. Fluorogold, a retrograde tracer, was used to locate the motor columns supplying the right sciatic nerve. Rats were killed by decapitation at 12 hr, 1d, 3d, 7d or 14d after sciatic nerve transection. All of these conditions were removed rapidly and frozen on dry ice. Longitudinal 20 μm sections were made through the lumbar spinal cord region containing the axotomized motor neurons. Using digoxigenin-labeled oligonucleotide or cDNA probes specific for each cytoskeletal protein, levels of mRNA expression were determined. These data suggest that message expression is down-regulated with advancing age, but that expression of cytoskeletal proteins after axotomy in the aging rat follows patterns similar to those seen in the young rat.

Supported by a grant to JMJ from the American Fed. for Aging Research.
710.21 EXPRESSION OF THE PRION PROTEIN (PrP) GENE IN CULTURED NORMAL HUMAN MUSCLE: E. Sako, W. Leiter, V. Askonas, J. McFerran, and W.K. Cai

Presented by A. Cookson and T. Liddle, University of California, Los Angeles, CA 90095-1699.

Even though abnormal accumulation of PrP has been considered unique to the brain, we have recently demonstrated abnormal accumulation of PrP and increased PrP-mRNA in cultured muscle fibers in inclusion-body myositis (reviewed by Askonas et al., 1994). PrP and its mRNA are also increased in human muscle fibers regenerating in vivo, but PrP-mRNA is virtually undetectable in the mature muscle fibers (except locally at the neuromuscular junctions). We have now studied the expression of PrP gene in cultured normal human muscle.

Adult human muscle was cultured aneuraly in monolayer from the satellite cells of portions of 5 normal muscle biopsies. PrP-mRNA abundance, relative to 28S ribosomal 20 and 40 days of growth by Northern blots, using 32P-labeled cRNA transcribed from a human PrP cDNA (gift from S.B. Prusiner). To monitor muscle development, creatine kinase (CK) activity was measured in situ cultures. At each stage of growth, cultured human muscle expressed strong PrP-mRNA. Its expression was lowest in 10-day-old cultures, and increased 144% (p < 0.02) in 20-day-old cultures. Between days 20 and 40, PrP-mRNA increased only 37% (p < 0.03). Between days 10 and 20, increase of CK activity was 166% (p < 0.04), similar to the increase of PrP-mRNA, but in contrast to PrP-mRNA, between days 20 and 40 CK activity continued to increase significantly 158% (p < 0.03) and linearly. Our study demonstrates for the first time that (1) the PrP gene is expressed in cultured normal human muscle; and (2) the highest level of its expression occurs during early muscle development. Thus, cultured normal and diseased human muscle should provide an excellent model to study factors influencing synthesis of PrP and its role therein.

LONG-TERM POTENTIATION: PHYSIOLOGY V

711.1 INHIBITION OF LONG-TERM POTENTIATION, BUT NOT LONG-TERM DEPRESSION, BY KYNAONE IN THE RAT DENTATE GYRUS Y. Wang, M. Suzuki, and R. Elde

Department of Physiology, Trinity College, Dublin, Ireland

Regulation of Ca release from the intracellular Ca stores of the endoplasmic reticulum (ER) is known to occur in part via the ryosynaptic receptor, a Ca-activated Ca channel located on the ER. The role of intracellular Ca stores in synaptic plasticity has been studied by investigating the effects of inhibiting the induction of long-term potentiation (LTP) and long-term depression (LTD). Intracellular patch clamp and field recordings of excitatory postsynaptic potentials and currents (EPSCs/EPSPs) were made from the dentate gyrus of hippocampal slices in response to stimulation of the associational/commissural pathway of rats (~100g).

In control recordings, LTP of EPSPs measuring 100±10% was detected. Following the administration of Kyn (20 min pre-stimulation) was induced in response to high frequency stimulation (8 trains each of 8 pulses at 200 Hz, 300 Hz, interval between 2 sec). LTD of field EPSPs and EPSPs measuring 40±5%, 32±5%, and 30±5% respectively was induced by low frequency stimulation (50 pulses at 1 Hz or 2 Hz). In experimental recordings, ryosynomine (20 μM) was applied to the perfusion solution in experiments recording field EPSPs or in the patch clamp electrode for recording EPSCs. Kynoidine was found to block LTP, but not LTD. Thus the amplitude of episodes at 20 min post-high-frequency stimulation in the presence of ryosynomine measured 99±5%, a significant inhibition of LTD. Low frequency stimulation induced LTD of field EPSPs and EPSPs measuring 34±6% and 40±6% respectively, values not significantly different from controls.

711.3 Ca2+ Signals Associated With The Induction Of Long-Term Potentiation And Long-Term Depression In Pyramidal Cells Of The Rat Visual Cortex. C. Hanel, A. Mehl, and M. Hergenhahn (SPON: European Neuroscience Association)

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We characterized Ca2+ signals evoked by tetanic stimulation patterns suitable for the induction of LTP and LTD in layer IIIa pyramidal cells of the rat visual cortex using the fluorescent Ca2+ indicator fura-2. Neuronal slices (200-250 μm thick) were obtained from 7-week-old rats. Sharp microelectrode recordings were used to measure responses to stimulation in either layer IV (conditioning pathway) or lateral layer II (control pathway). Three different plasticity patterns were established in which cells not filled with the indicator dye LTP was reliably induced by 5x repeated 50Hz stimulation in layer IV (8/9 cells). Application of only one 50Hz burst reduced the success rate in LTP induction to 50% (3/6 cells). Provided the repetitive 50Hz stimulation with a post-tetanic depression (PTD), there was no increase in PTD and a decrease in Mg2+ levels, leading to reliability of induction of LTD (6/7 cells). For characterisation of evoked Ca2+ dynamics, these tetanization patterns were applied to cells filled with fura-2 and changes of dendritic Ca2+ signals were measured with single-wavelength excitation at 340/380nm using an intensified CCD camera. Repetition of 50Hz burst (LTP pattern) increased burst-induced Ca2+ elevations, although Ca2+ levels very quickly recovered to baseline during the intervals between trains. LTP induction was sensitive to the single-train duration and frequency of stimulation. In contrast, Ca2+ levels accumulated during application of the LTP inducing tetanization pattern (5x) to levels higher than those measured during LTD induction. These observations indicate that the induction of LTD and LTP is associated with differences in evoked Ca2+ dynamics that are reflected by differences in both amplitude and duration of Ca2+ elevations. These results support the assumption that the different thresholds for the induction of LTD and LTP are related to the amplitude and the kinetics of the postsynaptic increase of [Ca2+].

711.2 CORRELATING DENDRITIC SPINE STRUCTURE WITH FUNCTION BY COMBINED ELECTRON MICROSCOPY AND Ca2+ IMAGING. T.H. Murphy, L. McAlister, M. Umemiya, and G.S. Knepper, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., V6T 1Z3.

By imaging Ca2+ entry associated with miniature excitatory synaptic currents (MESCs) under conditions that favor NMDA receptors (0 Mg2+, 5 mM Ca2+, and 1 μM TTX), we have been able to monitor the activation of a single synaptic site. Using this approach we concluded that the amplitude of repeated MESCs at a single synapse is proportional to the number of receptors available, furthermore some synaptic sites show a higher frequency of MESCs than others. Using cultured cortical neurons that were injected with fura-2 and biocytin, we evaluated the ultrastructural characteristics of dendritic regions that show these miniature synaptic Ca2+ transients (MSCTs) by peroxidase staining and serial sectioning. In evaluating the Ca2+ images we identified a presumed high probability region that exhibited 12 MSCTs from a single ~1 μm2 dendritic site. Neighboring sites were either inactive or showed only 1 MSCT. Transmission EM revealed that a single large spine (≈1 μm2) was centered at the site of MSCT initiation at the presumed high probability synapse. Other synapses were several μm away, making it unlikely that they contributed to the MSCTs. Our results suggest that a high frequency of synaptic responses of varying amplitude can be attributed to a single large synapse.

711.4 THE INTERPLAY OF DENDRITIC SPINE MORPHOLOGY WITH DEVELOPMENT OF GLUTAMATE RECEPTORS AND VOLTAGE-GATED CALCIUM CHANNELS AND ITS IMPLICATION FOR CALCIUM DYNAMICS. S.S. Dalpe and C.E. Niessen. Dept. of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089-1451; and Div. of Neurology, Children's Hospital of Los Angeles and USC School of Medicine, Los Angeles, CA 90027.

A detailed biophysical computer model that simulates calcium dynamics in dendritic spines in rat hippocampal neurons was created. Two features distinguish this model. Whereas previous models have only taken into account calcium efflux from the AMPA and NMDA glutamate receptors (GluRSs), the present model accounts for calcium influx into dendritic spines from both GluRSs and voltage-gated calcium channels (VGCCs). The existence of N-type VGCCs, within a subset of hippocampal spines, has been confirmed by confocal microscopy (Mills et al., 1994). The distribution of VGCCs and GluRSs on dendritic spines was varied with age from the first postnatal day (PN1) to adulthood as was dendritic spine morphology. Mechanisms that stimulate diffusion, pumping, and buffering of calcium were taken from the methods described by Holmes and Levy (Holmes and Levy, 1990). Results from the simulations are in very good agreement with calcium imaging studies in the hippocampal slice (Gubrian et al., 1991; Jaffe et al., and Comer et al., 1994) and other modeling studies (Gold and Bear, 1990; Kosh and Zador, 1993), i.e. presynaptic stimulation elevates calcium levels in the spine head to tens of micromoles while in the dendritic shaft, calcium concentration increases to less than 1 μM. In the adult, both the N-type and the T-type VGCC as well as the NMDA GluR are major contributors to this calcium boost which resulted in LTP. Neither the L-type VGCC nor the AMPA GluR alone could induce LTP. In modeling dendrites less than PN7, the lack of N-type VGCCs and the predominance of headless spines resulted in an absence of LTP. Both NMDA-dependent and NMDA-independent LTP can be induced in the model. Supported by USC Zumberg Research Award and NIH 1K08-NS01747-01.
711.5 INDUCTION OF LTD AND LTD OBSERVED SIMULTANEOUSLY WITH A ROSE OF POSTSYNAPTIC CALCIUM IN RAT VISCERAL CORDU.

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An input-associated increase in calcium at postsynaptic sites is suggested to trigger processes for the induction of long-term changes in synaptic efficacy. Furthermore, the extent of this increase is hypothesized to determine the direction of the changes, i.e., a large increase beyond a certain threshold leads to long-term potentiation (LTP) while a small increase below the threshold to long-term depression (LTD). This hypothesis seems testable with microfluorometry using calcium-indicators, such as fura-2 or fluo-3, but so far was not successfully probed at least in neocortex, probably because the near-simultaneous blocking action. So, we used another indicator, rhod-2, which has a much weaker chelating action. With this dye, we measured changes in calcium concentration in visual cortical neurons during tetanic synaptic inputs simultaneously with whole-cell clamp recordings of synaptic activity.

In visual cortical slices of young rats at 9–18 postnatal days, mod-2 was injected directly into pyramidal cell-like neurons of layer II/III through a patch pipette from which we simultaneously recorded excitatory postsynaptic potentials (EPSPs) of the neuron under observation. Tetanic stimulation of 4-burst type given to layer IV induced a clear increase in fluorescent signal. Usually, this increase was stronger in dendrites than in soma. Also, the delay time of the increased signal was much faster in dendrites than in soma. This was a tendency that tetanic stimulation which led to the induction of LTD of EPSPs induced a higher rise of calcium signal in dendrites than that which led to the induction of LTD.

711.7 MODELS OF CALCIUM BINDING TO KINETICALLY DISTINCT SITES ON CALMODULIN: IMPLICATIONS FOR LTD. D. V. Totty1,*, W. R. Holmes2, A. B. Christie3, D. Johnston4.

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We have revisited the Holmes & Levy (1990) biophysical model of associative LTD. Again we consider a morphologically correct granule cell of the dentate gyrus, with high-frequency synaptic activity (8 firings at 400 Hz), which causes Ca2+ influx through NMDA channels into a modelled spine. Here we update our model of calcium’s interaction with calmodulin to include the currently accepted kinetic model of this interaction. The calmodulin concentration used in the simulations was 20 μM. The four Ca2+ binding sites on a calmodulin molecule were modeled using the different kinetic constants summarized in Cox et al. (In Calcium and Calcium Binding Proteins, 1988) for the situation in which binding proteins are present. In these conditions, binding of the third Ca2+ to a calmodulin molecule exhibits a very high cooperativity (hβ). Brief, high-frequency activation of a single synapse produces 2-5 μM of calmodulin saturated with four bound calcium ions. This concentration (according to the model of Rios 256-1199, 1992, and assuming that there is more calmodulin than CaM kinase II) is enough Ca2+Calmodulin to fully activate CaM kinase II. This may even be enough Ca2+Calmodulin to lock the CaM kinase II holoenzyme in a permanent autophosphorylation mode. Thus, these data indicate that CaM kinase II activation alone will not be sufficient to produce associative LTD because, in vivo, single synapses are not sufficient for homosynaptic LTD.

Supported by NIH NS15488 and MH00622 to WBL.

711.9 FLUORESCENCE IMAGING OF INTRACELLULAR CALCIUM DURING THE INDUCTION AND EXPRESSION OF HOMOSYNAPTIC LTD IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. B. R. Christie*, J. Magee, and D. Johnston Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Hippocampal neurons exhibit various forms of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD). The induction of LTD depends, among other things, upon a rise in postsynaptic Ca2+. To investigate the changes in Ca2+ during LTD, we have combined in vivo imaging with whole-cell recordings of visually identified CA1 neurons. Hippocampal slices (600 μm) were obtained from 2–4 week old rats using standard procedures and the effects of brief, high-frequency synaptic activity were measured. In a subset of experiments, Ca2+ activity in a subset of experiments was monitored. Whole-cell recordings were made from CA1 neurons located within 50 μm of the slice surface. Following a 10 min baseline period, cells were synaptically activated at 2 Hz (20 stimuli per train) and changes in fluorescence were monitored. LTD, which lasted at least 30 min, only occurred in those neurons in which sufficient stimulation was given to elicit plastic synaptic poten-

tial changes during 3 Hz stimulation (threshold: > 7.1 ± 5.8, n = 3; supra-threshold: > 80.5 ± 5%, n = 4). During the application of the 3 Hz stimulation, only neurons in the supra-threshold group exhibited detectable changes in postsynaptic calcium levels (ΔF/F, soma: 7.4 ± 1.1, 0.5-7 μM: 6.9 ± 1.0, 7-150 μM: 6.2 ± 1.5% > 2.0% (n = 4). These events during subsequent synaptic stimulation are involved in the induction and maintenance of LTD (MH4474, MH48437, NS11039, NSERC10103).


Increase in the intracellular calcium concentration ([Ca2+]i) triggers both long-term potentiation (LTP) and depression (LTD) depending on stimulus intensity. Although mechanisms which underlie long-term synapic modification are yet to be elucidated, Ca2+ was found to play a key role in the present experimental study, we constructed a model for [Ca2+]i and Ca2+-binding proteins in a spine, and analyzed their dynamics. The present model includes N-methyl-D-aspartate (NMDA) and α-amin-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA) receptor channels with desensitization, Ca2+ influx through NMDA receptor channels, Ca2+-buffers, Ca2+- binding protein, Ca2+-extrusion, and Ca2+ diffusion. Dynamics of intracellular Ca2+ and Ca2+-binding protein for tetanic stimulation (100Hz), low frequency stimulation (1Hz), and theta burst stimulation were compared. The increase in [Ca2+]i was transformed when stimulation continued. This is because AMPA and NMDA receptor channels are desensitized, and the inward flow of Ca2+ is reduced. Furthermore, the peak level of [Ca2+]i was much higher in theta-burst stimulation than in tetanic (continuous stimulation) because of the slow recovery from desensitization. This result explains why theta-burst is effective for inducing LTD. In continuous stimulation resulted in smaller increase in [Ca2+]i which would lead to LTD. The concentration of activated form of calmodulin increased far more than the increase in [Ca2+]i, as stimulating intensity is increased. Therefore, the role of calmodulin is to amplify the Ca2+ signal and stabilize the differentiation between LTD and LTP.
11.11 A PARALLEL INCREASE OF NMDA AND NON-NMDA RECEPTOR-MEDIATED CURRENTS IN CA1 INHIBITED BY CA2+-INDUCED AMPA RECEPTOR BLOCKAGE. S.-N. Yang, D. W. Robinson and J. M. Horwitz. Soc. of Neurobiol., Physiol., and Behav., Univ. of Calif., Davis, CA 95616.

The report presents the establishment of Ca2+-induced LTP in area CA1 of the hamster hippocampal slice (Soc. Neurosci. Abs., 20, 119.3, 1994). Here, we further study the involvement of NMDA and non-NMDA receptor-mediated currents in LTP. Using photostimulation recording of excitatory postsynaptic currents (EPSCs) evoked by single shocks to Schaffer fibers, and action potentials induced by depolarizing current injections were made from CA1 pyramidal cells of hamster hippocampal slices (350µm). The average resting membrane potential ($V_{m}$) was 95 ± 5 mV. In current clamp mode ($V_{m}$ = 65 mV), application of high Ca2+ without stimulating Schaffer fibers only evoked a short-term increase of the firing rate of action potentials ($V_{m}$).

In contrast, a long-term increase of the firing rate was seen if sufficient single shocks were applied during a high Ca2+ pulse ($n$ = 2). In voltage clamp mode ($V_{m}$ = 60 mV), a high Ca2+ pulse induced a parallel, long-term increase of both NMDA and non-NMDA receptor-mediated currents of the dual component EPSC ($E_{p}$). Both the scaled EPSCs ($n$ = 3) and normalized amplitudes of the decay times of EPSCs prior to, and following Ca2+-induced LTP showed a similar pattern ($\gamma$).

In addition, following a stable Ca2+-induced LTP, tetanization of Schaffer fibers does not provide any additional enhancement of either NMDA or non-NMDA receptor component ($\nu$). These results suggest that Ca2+-induced LTP shares basic common mechanisms with tetanically-induced LTP. Moreover, the maintenance of Ca2+-induced LTP involves a similar increase in NMDA and non-NMDA receptor components and no change in decay times of EPSCs. [NASA Grant NAG2-2788]

11.13 DIFFERENTIAL EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS ON LTP AT BASAL AND APICAL SYNAPSES OF HIPPOCAMPAL CA1 NEURONS. J.E. Halley and D.V. Spritzer. Dept. of Molecular and Cellular Physiology. Stanford University School of Medicine, Stanford, CA 94304-5626.

Diffusible messengers such as nitric oxide have been proposed to act as intercellular signals in the production of long-term potentiation. Recent reports (Podolsky et al., 1994, Science 265, 342-6, Wendland et al., 1994, PNAS 91, 2511-5) illustrate that different forms of NOS are located to the somato and apical dendrites, but appear to be absent from the basal dendrites of those neurons, LTP elicited in synapses terminating in the basal dendrites of CA1 pyramidal cells may not depend on NOS, in contrast to apical synapses. We have tested this theory by comparing the effects of the NOS inhibitors aminoguanidine (in stratum radiatum (m.S.); apical) and 6-oxod-Glu (m.s.; basal). In control, tetanization (100 Hz/ sec) in the area of the NOS inhibited slice resulted in LTP of 154.8 ± 17.9% (n = 5). The same concentration of L-NAME (100 µM), identical tetanization in m.S. and control resulted in normal LTP (154.6 ± 17.9%) but following tetanization in m.S., LTP did not occur (111.1 ± 9.9%). Utilizing intracellular recording, we attempted to see if we could detect differences in NOS inhibitors when they were injected into single postynaptic cells. In these experiments, LTP was induced by pairing postsynaptic depolarization (by current injection) with Hz stimulation delivered simultaneously to the CA1 region of the NOS inhibited slice (n = 3). In control, this resulted in LTP of 155.7 ± 14.9% in m.S. and 138.1 ± 13.6% in s.o. After injection of L-NMMA (100 µM) to m.S. postynaptic cells LTP was prevented in both pathways (n = 3) 113 ± 9.06% (m.S. 100 ± 17.7%). Thus a different effect on LTP was not seen when using intracellularly injected NOS inhibitor with pairing-induced LTP. Present results suggest that there may be a differential role for nitric oxide in apical vs. basal dendritic synapses in area CA1 of hippocampus. However, not all experimental results are consistent with this hypothesis. This discrepancy is being investigated.

11.15 PHOTOLYTIC RELEASE OF NITRIC OXIDE BLOCKS INDUCTION OF LTP BY DEPRESSION OF NMDA RECEPTOR-MEDIATED TRANSMISSION IN THE CA1 REGION OF RAT HIPPOCAMPUS. K.P.S. Murphy and T.P. Bliss*. National Institute for Medical Research, Mill Hill. London NW7 1UA, U.K.

We have used flash photolysis of a caged form of nitric oxide (KbNOCaO3) to rapidly deliver known concentrations of nitric oxide (NO) to area CA1 of hippocampal slices (Sprague-Dawley rats, 200-250g). Photolysis was performed at 24°C. In previous experiments designed to test the retrograde messenger hypothesis for NO were unable to detect NO-mediated potentiation of synaptic efficacy (Murphy et al. 1994, Anesthesiology 32: 1377-1385). We report here evidence that NO may instead serve a role in setting the threshold for the induction of LTP (cf. figure 1). In area CA1 of the rat hippocampus (n = 10), tetanization of LTP was induced with 1.4±0.5mM NO (P<0.05 for effect of NO vs. control). These data indicate that NO, which has been shown to block the induction of LTP in area CA1, may also play a role in setting the threshold for LTP induction in this region.


The hypothesis that nitric oxide (NO) contributes to LTP has received considerable amount of experimental support. At the same time, many laboratories have presented evidence that inhibitors of nitric oxide synthase (NOS) fail to block LTP, or do so only under restricted conditions. These results suggest that NO might regulate the threshold for LTP induction at CA1 synapses. We have tested an alternative hypothesis; that NO might be required for LTP induction at CA1 synapses, but might not be required for LTP induction at CA1 synapses. According to this hypothesis, these synapses would be the most readily excitable of a heterogeneous group of CA1 afferents, and thus the most likely to be activated by weak stimulation. Therefore, LTP at the low end of the input/output curve should be blocked by NOS inhibitors, even following strong tetanus.

We have used an experimental protocol in which we alternated "weak" stimulation (40 µsec pulses) with "strong" stimulation (100 µsec pulses). Experiments were conducted at 31.2°C, in slices from rats taken from 15-150 days old. Stimulus intensities were adjusted so that strong stimulation elicited a response about 30% of the maximum evoked field EPSP (EPSP). After repetitive stimulation, the ratio of EPSP amplitude following the "weak" to the "strong" stimulus was measured. In the presence of the 100 µM NOx and NOx+NOx pulses (72/26), but not in the absence of 100 µsec pulses (103/68). Bath application of 100 µM NOx+NOx had no effect on baseline responses measured with 40 Mµsec pulses (991±1% of pre-drug baseline), or on LTP induced before drug application (150/250 at 60 min, 144/141 at 120 min). The results indicate a role for NO in LTP induced under physiological conditions, and suggest the presence of important heterogeneities in SOC afferents to CA1. Supported by NSF grant IBN-9410311.

Several studies have indicated that the induction of long-term potentiation (LTP) in the motor cortex may be dependent on the production of nitric oxide (NO). More recent studies have indicated that NO synthase inhibitors do not block LTP induction in all cases. To investigate whether NO is involved in the induction of activity-dependent synaptic plasticity in neocortex, we examined the role of NO in the production of LTP in rat motor cortex by administering extracellular NO synthase inhibitors. The results indicated that iontophoretic application of 50 mM N^nitro-L-arginine (NOARG) had no significant effect on the amplitude of the population spike (PS) and EPSP. A similar result was observed when 40 mM hemoglobin was applied. Even at high concentrations of NOARG (80 mM) and hemoglobin (60 mM), the amplitudes of the PS and EPSP did not change significantly. LTP was induced by tetanic stimulation (50 Hz) in the presence of NOARG (50 to 80 mM) and hemoglobin (40 to 60 mM). Neither of these treatments significantly affected the induction of LTP.

The results presented here indicate that, in contrast to the hippocampus, NO does not play a major role in induction or maintenance of LTP in motor cortex. NO synthase inhibitors in the motor cortex have failed to reveal any significant effect on both evoked responses and the induction of LTP. These results suggest that NO is not necessary for LTP induction in motor cortex. Supported by MH5321 and NSF BRS 852119.

711.18 β-AMYLOID ENHANCEMENT OF LTP AND NMDA-MEDIATED TRANSMISSION IN RAT HIPPOCAMPUS. Michael Rowe/James Wang and Roger Amyl Dept. Pharmacology and Therapeutics, Trinity College, Dublin 2, Ireland.

β-Amyloid is released at central synapses and may have a role in modulating glutamatergic synaptic function. We have investigated this possibility in the rat dentate granule cells. Monosynaptic slices were isolated and superfused using conventional techniques. Excitatory postsynaptic potentials and currents (epps and epscs) evoked by electrical stimulation of the mossy fibre inputs to the dentate granule cells were recorded with extracellular and intracellular (whole cell patch clamp) electrodes respectively. β-Amyloid (1-40) was bath-applied (200nM) and applied intracellularly (0.5μM). The receptor-mediated transmission was pharmacologically isolated using the AMPA receptor antagonist NBQX. Bath or intracellular application of β-amyloid resulted in a gradual enhancement of the NMDA receptor-mediated epsc which plateaued approx. 15 min later at a level averaging 140-160% of baseline (P<0.05). In those experiments where β-amyloid was washed off there was reversal of the effect. Although there was no effect on basal AMPA receptor-mediated transmission after bath application of β-amyloid, high frequency stimulation-induced long-term potentiation (LTP) of the field epsps was enhanced by 30% (P<0.05). The enhancement of LTP of AMPA receptor-mediated transmission indicates that β-amyloid can facilitate synaptic plasticity. The long-lasting increase in basal receptor-mediated transmission is direct evidence that β-amyloid selectively interacts with this potentially neurotoxic target via a postsynaptic site.

LONG-TERM POTENTIATION: PHYSIOLOGY VI

712.1 LONG TERM POTENTIATION OF THE SPINAL CORD MOTORNEURON DEMONSTRATED IN THE SPINAL CORD OF NEONATAL RATS. R.A. Nagell and Mary Chao Univ. of California, Davis, CA 95616.

Long term potentiation (LTP) is a used dependent form of synaptic plasticity characterized by a long lasting enhanced synaptic strength. In the hippocampus LTP appears to be the physiologic substrate of memory. The existence and possible role of LTP at the spinal motorneuron has not been fully demonstrated. We have used the immature spinal cord of rats (1-6 days old) isolated with recordings of ventral root (VRFPs) and simultaneous electrical stimulation of the dorsal nerve roots or corticospinal tract. Sequential short trains of tetanic stimulation at 100 Hz induced 3 seconds induced long-lasting potentiation of VRFP amplitudes. This phenomenon was partially and reversibly abolished by perfusate with 2-amino-5-phosphoovaleric acid (APV). Exposure of the spinal cord to the non-N-methyl-D-aspartate (non-NMDA) receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) completely and irreversibly abolished VRFPs. These findings suggest that LTP is present at the spinal motorneuron of neonatal rats. The partial obliteration of this phenomenon with APV suggest that LTP at the spinal motorneuron may involve the excitatory amino acid neurotransmitter system.

712.2 SUSTAINED POSTSYNAPTIC POTENTIATION OF MINIATURE EXCITATORY POSTSYNAPTIC POTENTIALS IN CULTURED RAT SYMPATHETIC NEURONS. K. Kubo, C.Liu, and T. Shimazaki, Dept. of Physiology, Saga Medical School, Saga 840, Japan.

Nicotinic synapses formed between cultured neurons of the rat's superior cervical ganglia were studied with whole cell patch clamp and fura-2 fluorescence recording techniques. A rapid rise in extracellular K^+ to 40-45 mM elicited a lamination of the NMDA receptor-mediated epsps (MEPSs).The amplitude of MEPSs gradually increased in 120-200mM during the course of a high K^+ treatment. On the other hand, the frequency fluctuated around a constant value occasionally with oscillation at a rate of several minutes. Furthermore, an accompanied rise in the intracellular Ca^2+ of putative presynaptic neurons was unchanged or gradually reduced under the same condition. The inward current induced by acetylecholine after the end of a high K^+ treatment was also augmented. Indicating the post-synaptic mechanism of MEPSs. The potentiation was seen for more than 20 min after the end of the high K^+ treatment and reversibly inhibited by phorbol ester (20 nM). Since the cytoplasm of the ganglion cell body was isolated through a patch pipette, the mechanism of potentiation may take place in the localized region of the postsynaptic membrane or may not involve a diffusible messenger.


We have investigated developmental changes in the size and density of dendritic spines on CA1 pyramidal cells in organotypic cultures using confocal microscopy. Cultures were prepared from three day old Wistar rats using a method adapted from Streppini et al. (J. Neurosci. Meth. 37, 173, 1991). At 5-7, 21-26 days, CA1 pyramidal neurons were stereotopically injected with Lucifer yellow dye, and imaged with a Biorad MRC 1000 confocal microscope. The architecture of CA1 cells from a mature organotypic culture was similar to that seen in CA1 neurons in acute slices from 3-4 week old rats. The stereologically uncorrected mean interval between spines on secondary apical dendrites was 1.4 ± 0.17 μm (n=80), and on basal dendrites 1.7 ± 0.13 μm (n=120); the mean observed spine length was 1.12 ± 0.04 μm (n=40) for apical dendrites and 0.80 ± 0.02 μm (n=40) for basal dendrites. These values are similar to those reported for CA1 cell cultures. In contrast, spine density from immature cultures (<1wk) was significantly less (mean density: 2.07 ± 0.26 μm (n=33) for apical dendrites; p < 0.01; and 2.35 ± 0.37 μm (n=33) for basal dendrites; p < 0.01). The length of spines however, was not significantly different from that observed in mature slices (mean length = 1.0 ± 0.06 μm (n=37) for apical spines (N.S.), and 1.10 ± 0.07 μm (n=40) for basal spines (N.S.)).

These results indicate that CA1 neurons in organotypic hippocampal cultures systemically add spines during development, so that the number of spines that is achieved is comparable to that observed in acute slices, despite the fact that there are fewer CA3 projection cells in organotypic cultures than in the intact animal from which acute slices are cut. This implies either that the number of CA3 cells projecting to a given CA1 cell is greater in organotypic cultures than in acutely prepared hippocampal slices, or that there is a much higher number of contacts per target cell. The physiological observations of Debanne et al. (J. Neurophysiol. 73, 1782, 1995) indicate that the former is primarily the case.
DENDRITIC SPINES IN HIPPOCAMPAL NEURONS: CORRELATING STRUCTURE AND FUNCTION. C. Collins 1, K. Miyashita2, and M. Segal1, 1LAS, TN. L, Bangkok, 2VNSCI, Tokyo, Japan. Weizmann Institute, Rehovot 76100, Israel.

Dendritic spines have been proposed to constitute the locus of long-term memory storage in central neurons, yet little is known about the biophysical and morphological mechanisms underlying their role in memory. We studied the effects of tetanic stimulation on presynaptic transmission on spine morphology and ability of spines to express long-term potentiation (LTP) of population responses to different stimulus in area CA1 of rat hippocampal slices maintained in culture conditions for 1-5 weeks. Blockers of excitatory or inhibitory neurotransmission were added to some cultures. Extracellular field EPSPs (fEPSP) were recorded in field CA1 in response to stimulation of Schaffer collaterals, which were labelled with Lucifer yellow, and were subsequently visualized in a confocal laser scanning microscope. Within 10 days in culture, CA1 dendrites had lost filopodia-like structures and almost no spines. fEPSP amplitude was 3.5±1.1mV and tetanic stimulation (100Hz, 1 sec) caused no LTP. At 3 weeks, spines were present in large heterogeneity (mean density=0.81 spines/mm of dendrite), but the fEPSP amplitude was 5.6 ± 0.9 mV, and tetanic stimulation caused LTP in 63% of the slices. At 5 weeks, spine density was higher, but no significant increase in the fEPSP amplitude or ability to produce LTP was observed. Slices grown in the presence of picrotoxin did not show changes in spine density at 3 weeks, but the fEPSP amplitude was significantly smaller than controls (1.9 ± 0.6 mV). Tetanic stimulation failed to induce LTP. Spine density was reduced in slices grown in the presence of the NMDA antagonist APV. fEPSP amplitude was same as controls, but LTP was induced in 100% of the slices. The data support that the maturation of dendritic spines in hippocampal neurons is age-and activity-dependent. The ability to express LTP is correlated with the appearance of spines, but not with variations in spine morphologies.


We have begun to compare the properties of long-lasting potentiation in dissociated hippocampal cell culture with LTP in slices. Pairs of pyramidal-shaped neurons were maintained throughout the experiment under whole-cell voltage clamp. Three high-frequency trains of depolarization (2.5 sec, 2 Hz) of the presynaptic neuron in O-Mg2+-saline produced immediate potentiation of the EPSC that lasted until the end of the experiment (average = 5% increase, p < 0.1, n = 11). There was no potentiation in experiments in which the bath solution contained the NMDA receptor blocker APV (50 μM) or in which the tetanic stimulus amplitude was reduced to 75% of the control (n = 12). We were able to produce long-lasting potentiation in normal Mg2+-saline, but, however, by pairing low-frequency stimulation of the presynaptic neuron (1 Hz for 30 sec) with depolarization of the postsynaptic neuron to O-mV (average = 48% increase, p < 0.1, n = 8). Potentiation by tetanic stimulation was blocked by injection of the Ca2+ chelator BAPTA (20 mM) into the postsynaptic neuron (n = 7). Potentiation was also blocked by injection of the NO synthase inhibitor N-methyl-L-arginine (0.5 mM) into the postsynaptic (p = 9) but not the presynaptic (p = 8) neuron. Conversely, long-lasting potentiation could be produced by bath application of NO (10 mM) paired with weak tetanic stimulation (50 Hz, 0.5 sec) of the presynaptic neuron (average = 37% increase, p < 0.02, n = 10). Finally, potentiation by either tetanic stimulation (n = 9) or low-frequency stimulation paired with postsynaptic depolarization (n = 8) was blocked by bath application of the G kinase inhibitor Rp-8-Br-GMPS (100 μM).


Tissue plasminogen activator (tPA) has been implicated in a number of cellular processes in the nervous system such as axonal elongation and cell migration. Qian et al. (Nature 361:453, 1993) have demonstrated that long-term potentiation (LTP) increases tPA expression in the dentate gyrus. We have found that forskolin, an adenyl cyclase activator, which produces LTP in the mossy fiber pathway to CA3 pyramidal cells (Huang et al., Cell 79:69, 1994), induces a five-fold increase in tPA mRNA levels in cultured neurons within 10 min of forskolin treatment. This forskolin-induced expression of tPA in hippocampal cultured cells is comparable to CA3 pyramidal cells (Huang et al., Cell 79:69, 1994), induces a five-fold increase in tPA mRNA levels in cultured neurons within 10 min of forskolin treatment. This forskolin-induced expression of tPA in hippocampal cultured cells is comparable to CA3 pyramidal neurons. In addition, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Furthermore, we have found that forskolin increases expression of tPA in hippocampal cultured cells. Further-
LONG-TERM POTENTIATION: PHYSIOLOGY VI

T12.11


It has recently been shown in hippocampal LTP that mossy fiber (MF) synapses are induced and expressed presynaptically and may be mediated by KA receptors (Ehlers, et al., 1994). The MF to entry during a tetanus is proposed to activate a Ca sensitive adenyl cyclase (AC), leading to an increase in cAMP. This increase, via PKA can alter cell functions that decrease glutamate release. Because AC is highly expressed in parallel fibers (PF) we examined whether a similar form of LTP occurs at parallel fiber-Purkinje cell synapses in rat cerebellum. Using PF silencing recording techniques enabled us to monitor simultaneously both the fiber volley and postsynaptic response. Low frequency stimulation (LFS; 4 Hz for 20 sec) caused a decrease in the field amplitude of the EPSP that lasted at least 1 hour. In most cases the fiber volley was only modified transiently after LFS, but in some cases was enhanced over a prolonged period of time. PF-LTP was associated with a decrease in paired pulse facilitation (PPF) and application of the glutamate receptor antagonist kynurenic acid (10mM) during LFS of PF did not prevent the induction of LTP. These results indicate that PF-LTP is presynaptic in induction and expression, as previously suggested in cultured cerebellar neurons. Furthermore, a short term application of the adenosine cyclic activator forskolin caused an enhancement of PF synaptic responses that lasted at least 1 hour and induced a decrease in PPF. These experiments strongly suggest that common mechanisms are involved in PF and MF LTP. Supported by the NIH.

T12.13

Spatial localization of current sinks in the superficial layers of the entorhinal cortex following photostimulation in the piriform cortex. C.M. Chapman* and RJ Raisig. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S-4K1.

Field potentials in rat entorhinal cortex were potentiated for very long periods following strong stimulation of the piriform cortex (SN Abstr 20.585.2), but the synaptic populations underlying these effect could not be spatially localized. Biphasic field pulses (225 to 650μA) were delivered to the piriform cortex (AP: 3.6, L65, V6.5-10mm) of urethane anesthetized (1.5g/kg) rats. A stainless steel barrel containing a platinum electrode was advanced on the sagittal plane, 40° off horizontal and perpendicular to entorhinal cortex laminar, and aimed at a point 5.2mm in depth and 26.2mm lateral to the ascending line. The average of 10 evoked field potentials were recorded at 50μm intervals at depths between 0.0 and 2000μm. The evoked field potentials were an excellent filter of PF synaptic responses that lasted at least 1 hour and induced a decrease in PPF. These experiments strongly suggest that common mechanisms are involved in PF and MF LTP. Supported by the NIH.

T12.14

Low-frequency stimulation potentiates LTP in rat piriform cortex. W. Krauh and D.O. Carpenter. Department of Pharmacology, University of Washington, Seattle, WA 98195 USA.

Field potentials in rat entorhinal cortex were potentiated for very long periods following strong stimulation of the piriform cortex (SN Abstr 20.585.2), but the synaptic populations underlying these effect could not be spatially localized. Biphasic field pulses (225 to 650μA) were delivered to the piriform cortex (AP: 3.6, L65, V6.5-10mm) of urethane anesthetized (1.5g/kg) rats. A stainless steel barrel containing a platinum electrode was advanced on the sagittal plane, 40° off horizontal and perpendicular to entorhinal cortex laminar, and aimed at a point 5.2mm in depth and 26.2mm lateral to the ascending line. The average of 10 evoked field potentials were recorded at 50μm intervals at depths between 0.0 and 2000μm. The evoked field potentials were an excellent filter of PF synaptic responses that lasted at least 1 hour and induced a decrease in PPF. These experiments strongly suggest that common mechanisms are involved in PF and MF LTP. Supported by the NIH.

T12.15


We examined the possibility to induce long-term changes in synaptic transmission in the visual cortex by applying bursts of intracellular stimulation pulses (intracellular tetanization, IT) to the postsynaptic cell, without concomitant presynaptic stimulation. Posttetanization potentials evoked from two stimulation sites in layers II and I-III cells with sharp whole cell electrodes. IT led to marked changes in synaptic transmission in the majority of cells. Para-2 based Ca2+ measurements showed that IT produced a substantial increase in [Ca2+]i, comparable in magnitude to increases evoked by strong afferent tetanization. Inputs from layer IV were more often potentiated while layer II inputs were more often depressed. This difference is probably due to the different localization of the presynaptic affrents at the dendritic tree. Ca2+ imaging showed that stimulation in layer IV activates inputs to the proximal parts of the apical dendrites, while stimulation in layer II activates inputs to the distally located inputs. To evaluate the involvement of presynaptic mechanisms in the lasting changes of synaptic transmission induced by IT, we analyzed paired-pulse interactions before and after potentiation. Intracellular (PP) stimulation usually decreased paired-pulse depression (PDP) while the induction of long-term potentiation (LTP) and the magnitude of the decrease correlated positively with LTP magnitude and with the presynaptic PPF ratio.

Our data suggest that the potentiation of IT in the visual cortex, which lead to a significant rise of intracellular [Ca2+]i are capable to produce long-term changes in synaptic transmission in the visual cortex. Changes in PPF ratio after LTP induction of IT are potentiated and the presynaptic mechanisms are involved in the maintenance of synaptic modifications induced by IT.

T12.16


We have recently found that long-term potentiation can be induced in the nociceptors of chronically prepared rats, but only if the stimulation is applied over multiple sessions. These experiments were done with both stimulation and recording electrodes in the same hemisphere. The present experiments were done in rats in which a single train of noncontrollable LTP could be induced if the stimulation and recording electrodes were in homologous sites in opposing hemispheres. We also tested the effects of low frequency stimulation trains. These have been reported to induce depression effects in the hippocampus and visual cortex. Thirty, 24, 500 Hz trains were applied to the nociceptors for each day for 10 days. Stimulation intensity was set at 125μA. In one group, the trains were applied to the somatosensory cortex and in the other group, the trains were applied to the visual cortical. Control groups were also run for each site. Before and after the application of the posttetanization trains, pulses were applied to the stimulation site and the responses evoked in the contralateral site were monitored and analyzed. One week following the induction of LTP, 11 trains were applied to the stimulation site for 15 min after which the test pulse trains were run. We found that the baseline response amplitudes were much larger in the posterior sites, but the LTP effects were similar in both sites. The potentiation had shown little decay 1 week following the completion of the trains. At this point the latency of the responses showed increased HFs from each site. A transient depression effect was found following low frequency stimulation of the anterior site. These responses had returned to their baseline levels by 24 hours. The depression effect following stimulation of visual cortex was still present 7 days post-treatment. The control animals showed only minor depression effects in their baseline responses.

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117.18

LONG-TERM POTENTIATION IN THE CHICK IMHV REGION: E. Shimabukuro, K. Kyosue, M. Kasa, T. Taguchi, Dept. of Biophysics, Engineering, Sci., Osaka Univ., Toyonaka 560, Japan. (Supported by the Japan Society for the Promotion of Science and the Ministry of Education, Science and Culture of Japan.)

The intermediate and medial part of hyperstriatum ventral (IMHV) is critical part of chick forebrain to establish imprinting and many types of learning. Although many electrophysiological studies have been performed by using conventional patch-clamp technique, we have analyzed long term potentiation (LTP) in IMHV region, where E.P.S.C.s were evoked by local stimulus near the postsynaptic cell. The stimulus of 5 Hz for 1 min paired with postsynaptic depolarization was used to induce the potentiation. The potentiations were sustained for 80 min at most. The coefficient of variation of peak amplitude of E.P.S.C. did not change so much after the induction of potentiation. If the probabilistic transmitter release is governed by binomial process, this does not indicate increase of transmitter release probability but increase of postsynaptic efficacy. Further, the ratio of AMPA/kainate type glutamate receptor-mediated currents to NMDA type glutamate receptor-mediated currents changed after conditioning stimuli. These results indicate that postsynaptic change is included in the mechanism for LTP expression in this area.

117.19

SYNAPTIC PLASTICITY IN THE DIRECT FEEDBACK PATHWAY TO THE ELECTROSENSORY LATERAL LINE LOBE OF A. LEPTOrittVHY-VHIS. D. Wang, L. Maler and E. Detrick, Dept. of Anatomy and Neurobiology, University of Ottawa, Ottawa, Ontario, Canada. K1H 8M5

Electroreceptor afferents terminate in a laminated chemostructure: the electroreceptive lateral line lobe (ELL). In addition to its peripheral input, the ELL also receives feedback input: feedback reaches the molecular layer of the ELL via a direct feedback projection, the stratum fibrosum (SF) which terminates in the ventral molecular layer (VML). Earlier studies have revealed that the feedback projections were glutamatergic and associated with NMDA receptors. Here we report the electrophysiological properties of these synaptic currents including their dependence on synaptic input, and an increase in the amplitude of the late component (long-term to peak range: 5±6.8 ms) of the EPSP, an increase in the amplitude and number of population spikes, and a decrease in the incidence of the early component (from 92±8% to 9±3 ms). In many animals this late component is clearly seen only following potentiation. These potentiation measures increased over the course of trains in all animals. Animals receiving 60 trains/day required fewer trains to reach asymptotic levels of potentiation of the late component and displayed both a larger increase and subsequent decrease of the early component. In addition, these data are currently being collected and preliminary observations indicate that the groups receiving 60 trains/day have the longest LTP decay time constants. These chronic data provide much needed evidence that a postsynaptic output of long-term memory is capable of supporting LTP effects that can last for weeks or months.

117.20

HUMAN CORTEX SHOWS ROBUST NMDA DEPENDENT LONG TERM POTENTIATION. J.L. Lopez, W. Chen, C. Yau, D.D. Spencer, G.M. Shepherd and A. Williams, Sections of Neurosurgery and Neurobiology, Yale University School of Medicine, New Haven, CT 06520

LTP has been extensively studied in animal models but not in the human cortex. Here we report the induction of LTP in human neocortex, and its dependence on NMDA receptors. The tissue was obtained from temporal lobectomies for control of intractable epilepsy. We harvested pathologically and histologically normal cortical specimens from four patients. 500 μm slices were prepared and maintained in an interface chamber. EPPS were evoked by a train of 18 pulses at 180 Hz; LTP was induced by giving five trains of tetanic stimulation (100 Hz for 1 sec each) at 30 sec intervals. The EPPS amplitudes were measured for 30 min pre-tetanus and 60 min following five trains of tetanic stimulation in layer IV. LTP was induced by giving five trains of tetanic stimulation (100 Hz for 1 sec each) at 30 sec intervals. The EPPS amplitudes were measured for 30 min pre-tetanus and 60 min following five trains of tetanic stimulation.

In total, we performed 28 trials using these parameters in 17 slices. LTP was produced in 11 of 18 trials performed in normal ACSF. Averaged data from these 11 trials showed a 29±6.9±5.9% potentiation of EPPS amplitude 10 min. after the tetanus, and a 34.5 ± 6.6±4% potentiation 55 min after the tetanus. The remaining 7 of 18 trials showed no potentiation of EPPS amplitude. Activity-dependent synaptic plasticity was modulated but not eliminated by NMDA antagonists, APV. In 10 trials following the bath application of 50 μM APV, LTP was only seen in 2 of 10 trials. Chi Square analysis showed a highly significant effect of APV in decreasing LTP induction (P<0.0001).

Our study shows that LTP can be induced in adult human neocortical slices without the application of bicuculline. The incidence of LTP (61%) is comparable to that seen in the sensory cortex of young rats. We found that the incidence of LTP is significantly decreased by the application of NMDA receptor antagonist APV, suggesting that NMDA receptors are also involved in the plasticity seen in human neocortex.

LIGAND-GATED ION CHANNELS III

713.1

PROTEIN KINASE A OR PROTEIN KINASE C DOES NOT APPEAR TO BE INVOLVED IN THE INHIBITION OF ATP-ACTIVATED CURRENT BY ETHANOL IN SENSORY NEURONS. Chao Ling*, J. Zhai and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, National Institutes of Health, Bethesda, MD 20892-8205.

We have previously demonstrated that ethanol, in pharmacological concentrations, produced concentration-dependent inhibition of ATP-activated inward current in freshly isolated bullfrog dorsal root ganglion neurons by interacting with a small hydrophobic pocket on the receptor protein, rather than with membrane lipids (Proc. Natl. Acad. Sci. USA 91: 8200, 1994). Using the perforated-patch recording technique (nystatin 150 mg ml⁻¹ in the recording pipette), we found that the inhibition of the current produced by 2.5 μM ATP was decreased by the extracellular application of 100 mM ethanol by 40 ± 5% (n = 8 cells). This degree of inhibition was similar to that observed in the presence of ethanol using whole-cell patch-clamp recording. The inhibition was not significantly altered by the extracellular application of 100 mM ethanol to the bath. We investigated the possible involvement of protein kinase A and protein kinase C in the inhibition by using protein kinase inhibitors. [N1(N616R,N616Q)] obtained from Xenopus oocytes expressed in a two-electrode voltage-clamp method. Recordings were performed in Mg²⁺glycerol (10 mM) supplemented with 2 mM sodium pyruvate/1 mM ATP (PFA). Cells were clamped at -80 mV and currents were recorded in the presence of 100 μM Na⁺ and 10 mM Na⁺/Mg⁺ with varying concentrations of ethanol. Mn²⁺/Zn²⁺ to NR1 subunits (N116R) were treated for their sensitivity under different ionic conditions. The wild type and mutant NR1 subunits were expressed along with the N2A and the N2C in Xenopus oocytes. NMDA-induced currents were measured using solution-change methods. Recordings were performed in Mg²⁺/free normal frog ringer with 1.8 mM Mg²⁺ (Na-NF). ATP and 100 μM ATP was supplemented with 2 mM sodium pyruvate/1 mM ATP (PFA). Cells were clamped at -80 mV and currents were recorded in the presence of 100 μM Na⁺ and 10 mM Na⁺/Mg⁺ with varying concentrations of ethanol. Mn²⁺/Zn²⁺ to NR1 subunits (N116R) or NR1A or NR1B or NR1C in Na-NF or PFA were similarly sensitive to inhibition by ethanol when compared to the mutant NR1A receptors. Together, these results suggest that C²⁺ permeability of NMDA receptors may influence the sensitivity of the inhibition of current by ethanol. Supported by NIAAA 08059 and NIDA 07027.
113.4


Xenopus homomeric GLIC neurons express a higher density of GABA-A receptors that are differentially affected by ethanol. The role of GABAergic mechanisms in the action of ethanol in the brain has prompted interpretations concerning the role of alcohols in the development of dependence. To study the effects of ethanol on GABA-induced chloride currents, we used the patch-clamp technique to record chloride currents in Xenopus oocytes expressing GABA-A receptors. Activation of the chloride current by ethanol requires the presence of GABA, and can be detected at a GABA concentration of 0.1 to 0.5 µM. The concentration of ethanol required for measurable augmentation of the GABA-induced current was 5 mM and maximum augmentation was observed to occur at approximately 60 mM. In addition, only 2% of neurons responding to GABA also responded to ethanol and GABA, independent of concentration of either compound, the diameter of the neuron or the age of the animal.

The implications of these results are that 1) the effect on the chloride current by ethanol in hippocampal neurons is GABA dependent; 2) the effects are dependent upon specific properties of the individual neuron - most likely subunit dependent and 3) these concentrations are well within the range noted to induce a physiologic effect in animal models. Thus induced hyperpolarization of hippocampal neurons may be an important factor in the physiologic effects of ethanol.

113.5


We examined the effect of ethanol on glycine-activated currents (1-Gly) of hypothalamic (VMH) and cortical neurons freshly isolated from young mice. 1-Gly of 18 VMH and 29 cortical neurons was recorded with the whole cell patch technique. Holding potential was -60 mV and a fast superfusion system delivered varying concentrations of glycine (Gly) alone or in combination with 21.6, 43.2, or 64.8 mM ethanol. Peak 1-Gly was -227.04±71.06 pA (mean±SEM) for 5 VMH neurons exposed to 100 µM Gly. When 64.8 mM ethanol was co-applied to these neurons, 1-Gly increased to -471.0±134.84 pA. In contrast, for two other VMH neurons exposed to 100 µM Gly and 64.8 mM ethanol, 1-Gly decreased. Likewise, two of three cortical neurons were detected following exposure to 43.2 mM ethanol; peak 1-Gly increased and decreased for 2 neurons out of 4 examined. Similar results were obtained from VMH neurons exposed to 244 µM Gly and 21.6, 43.2, or 64.8 mM ethanol. 1-Gly decreased in all cortical neurons exposed to 100, 200 or 304 mM Gly. These differential responses of 1-Gly to relevant concentrations of ethanol suggest that while two populations of Gly receptor exist in the VMH, the cortex is homogeneous in this regard. Supported by grants NIAAA AA00252 and NIH NS10440.

113.6

ETHANOL INHIBITS RECOMBINANT α7 NICOTINIC ACETYLCHOLINE RECEPTOR-MEDIATED CURRENT IN XENOPUS OOCYTES. J. M. Kaiser, K. E. Isembera, C. J. Zorumski, N. T. Rodriguez-Neune*, and E. Weight#. Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The α7 nicotinic acetylcholine receptor (nAChR) subunit has been cloned and characterized; it has the ability to form homomeric channels when expressed in Xenopus oocytes (Neuron 5:33-48, 1990). In addition, it is found in the mammalian CNS. Because of its high permeability to Ca²⁺, it has been suggested that this subunit may be involved in the phenomena of neuronal plasticity, neural development and learning. Previous studies have shown that ethanol can potentiate nAChR mediated responses at the neuromuscular junction (J. Physiol. 244:409-429, 1975). Little is known, however, about the effects of ethanol on neural nAChR's.

We expressed homomeric α7 nAChR cRNA in Xenopus oocytes and studied the effect of ethanol on the function of these receptors using the two-electrode voltage-clamp technique. We found that ethanol concentrations from 10 mM to 100 mM could produce a concentration-dependent decrease in the amplitude of α7 nAChR-mediated current. Maximal inhibition was observed at an ethanol concentration of 100 mM; maximal inhibition was 36% of control current induced by 10 mM (-)-nicotinic acid. The EC50 was 50 mM and the apparent Hill coefficient was 1.6. The observations indicate that ethanol modulation of α7 nAChR-mediated current differs from the effect of ethanol reported previously for nAChR at the neuromuscular junction.

113.7

ALIPHATIC ALCOHOLS EXHIBIT A CUTOFF IN POTENCY FOR ENHANCEMENT OF GABA-ACTIVATED ION CURRENT: ROBERT W. PEOPLES* and FERREN F. WEIGHT. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892.

Intoxicating potency of straight-chain aliphatic alcohols increases as the number of carbons are increased up to 6 - 8 (the "cutoff"), then decreases despite increasing lipid solubility and membrane disordering potency (J. Pharmacol. Exp. Ther. 218: 669, 1981; Neuropharmacol. 17: 451, 1978). We have previously demonstrated that n-alcohols also exhibit a cutoff for inhibition of ion current activated by N-methyl-D-aspartate (NMDA) at 6 - 8 carbon atoms (Proc. Natl. Acad. Sci. USA 92: 2825, 1995). In this study, we investigated whether a cutoff for potentiation of GABA-A receptors by n-alcohols could be observed in mouse hippocampal neurons in primary culture using the whole-cell patch-clamp technique. We found that the potency of alcohols for enhancing GABA-A current was performed as the carbon chain length was increased up to 11 - 12 carbons, but that maximally-attainable concentrations of alcohols with more than 12 carbon atoms did not enhance GABA-activated current. This cutoff thus differs from those previously reported for behavioral measures of alcohol intoxication.

113.8

EFFECT OF PENTANOL ON RECOMBINANT ACETYLCHOLINE RECEPTOR CHANNELS EXPRESSED IN HEK-293 CELLS. A. Ravindran*, K. E. Isembera and E. Weight. Laboratory of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The nicotinic acetylcholine receptor (AChR) of adult skeletal muscle is a channel-forming glycoprotein consisting of four homologous subunits assembled into a β3β4 tetramer. The modulatory effects of 1-pentanol on acetylcholine (ACh)-induced current was studied in HEK-293 cells transiently transfected with mouse β4A, AChR subunit cDNAs, 30-40 hr after transfection whole-cell and outside-out patch-clamp techniques were used to record ACh-induced inward current from cells expressing AChR. ACh alone and in combination with different concentrations of 1-pentanol were applied to the recorded cell or outside-out patch using a rapid perfusion system that enabled complete exchange of solutions in 0.8-4 ms. ACh activated rapidly desensitizing inward currents with an EC50 of 13 µM. Co-application of 1-pentanol (0.5-16 mM) increased the amplitude of current activated by 2 µM ACh in a concentration-dependent manner. The potentiation was between 28-150% of the control response. The rate of ACh desensitization was also significantly increased (3-5 fold) by pentanol in a concentration-dependent manner. At agonist concentrations ≥ 5 µM, low concentrations of pentanol caused potentiation and high concentrations caused inhibition of the peak current. Further studies are underway to elucidate the mechanism of pentanol actions on AChR channels.
**713.9**

**INTERACTION OF GLYCINE AND ETHANOL ON RECOMBINANT HETEROLOGOUS NMDA RECEPTOR SUBUNITS.**

K. Masood*, F. E. Weight and C. Wu, Lab. Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

Glycine, a component of NMDA receptor channels, has been shown to potentiate NMDA-activated ion currents in heteromeric combinations. There is controversy regarding whether glycine can reverse or potentiate the effects of NMDA receptor channels. To address this issue and to understand the mechanism of action of ethanol at a molecular level, we examined the interaction of glycine and ethanol on recombinant eCl5, eCl3, and eCl1 subunit combinations expressed in Xenopus oocytes. Membrane current activated by NMDA was recorded using the two-electrode voltage-clamp technique with holding potential of -70 mV. Ethanol (10 mM) decreased E<sub>V</sub> of the glycine concentration-response curve for all four heteromeric combinations. The inhibition of E<sub>V</sub> by 100 mM ethanol for eCl1, eCl2, eCl3, and eCl4 combinations was 43%, 15.0%, 19.1 and 26.9%, respectively. However, ethanol did not significantly affect either the EC<sub>50</sub> or the apparent Hill coefficient of these curves (ANOVA, p>0.05). The results indicate that ethanol is not competitive with glycine for the eCl1, eCl2, eCl3 and eCl4 subunit combinations.

**713.10**

**ALIPHATIC N-ALCOHOLS EXHIBIT A CUTOFF IN POTENCY FOR THE INHIBITION OF RECOMBINANT GLU3 RECEPTOR SUBUNIT CURRENT IN XENOPUS OOCYTES.**

B. Emmanuel Akinbiola* and Forrest F. Wright, Lab. Molecular and Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The potency of straight-chain aliphatic alcohols for affecting the function of AT8, NMDA- and 5-HT3 receptor gated ion channels increases as the number of carbon atoms are increased up to a point called the "cutoff", where the potency decreases despite increased hydrophobicity of the alcohol (PNAS 91: 5290; 1994; PNAS 92: 2513; 1995; Soc. Neurosci. Abstr. 20: 1127, 1994). Although non-NMDA excitatory amino acid receptor-mediated responses have been reported to be inhibited with increasing potency with short-chain aliphatic alcohols due to membrane penetration (JPH 262: 487, 1992), it has not been determined whether non-NMDA receptors exhibit a cutoff phenomenon. To address this question, we used the two-electrode voltage-clamp technique to study the effect of a series of straight-chain aliphatic alcohols on recombinant AMPA-type GluR3 receptor subunits expressed in Xenopus oocytes. We found that for short-chain alcohols from methanol to hexanol, potency for inhibition of kainate-activated current increased in proportion to the chain-length or hydrophobicity of the alcohol. However, potency decreased with octanol and nonanol and did not inhibit kainate-activated current. The observations indicate that straight-chain aliphatic alcohols exhibit a cutoff in their potency for inhibition of non-NMDA glutamate receptors.

**714.1**

**SODIUM CHANNEL DENSITY IS INCREASED IN RAT DORSAL ROOT GANGLIA FOLLOWING CHRONIC CONSTRUCTION INJURY.**

J. Kwan, J. L. Riker, G. Coleman, F. Filloux*, J. Haven, and E. C. Koehn, Department of Neurosurgery, Mount Sinai Hospital, New York, NY 10029.

Neuropathic pain caused by peripheral nerve injury is associated with a persistent spontaneous discharge of injured fibers. This abnormal firing may be due to an accumulation of Na channels in peripheral sensory nerve fibers. To test this hypothesis we have used quantitative [H]tetrodotoxin ([H]TTX) autoradiography to examine the distribution, density (B<sub>max</sub> and affinity (K<sub>d</sub>) of Na channels in rats following CCI. Brains, spinal cords and dorsal root ganglia (DRG) were harvested from 6 normal rats and 6 rats 30 days after unilateral CCI. [H]TTX autoradiography was performed on 20 µm sections through cortex, midbrain periaqueductal gray, lumbar spinal cord, and L-4 DRGs. Saturation measurements were performed to model a single class of binding sites with 3<sub>1</sub> ranging from 0.7-3.9 nM and B<sub>max</sub> from 157-720 fmol mg<sup>-1</sup> tissue equivalent (i.e.). Na channel density was increased in the DRG ipsilateral to the nerve injury (B<sub>max</sub> 248±1 14.4 fmol mg<sup>-1</sup> i.e.) compared with the sham side (205.7 ± 7.9) or normal DRG (190 ± 20.5) (p<0.05). No differences were found in other regions. In summary, CCI caused an increased [H]TTX binding sites in DRG neurons that persisted for 30 days after the injury. This reflects an increase in the density of STX-sensitive Na channels, and suggests a mechanism for the effectiveness of sodium channel blockers in neuropathic pain.

**714.2**

**REGULATION OF SODIUM CHANNEL mRNA IN A NEUROPATHIC PAIN MODEL.**

S. G. Delgado and P. C. Holland, Department of Neurology, University of Michigan, Ann Arbor, MI 48109; and G. M. LaMotte and R. W. Bayliss, Biotechnology Unit, Syntex Research, Palo Alto, CA 94304.

To investigate the sciatic nerve evokes spontaneous electrical activity at high frequency. We have used a rat model where the sciatic nerve is loosely ligated to investigate the regulation of sodium channel mRNA levels. A neuropathic pain condition causes a unique profile of sodium channel mRNAs. The sodium channel mRNAs are expressed in DRGs in a ligature. The sodium channel mRNAs are expressed in DRGs in a ligature.

**714.3**

**PROLONGED O2 DEPRIVATION ALTERS Na+ CHANNEL mRNA LEVEL IN EXCITABLE TISSUES IN THE DEVELOPING RAT.**


We have previously observed an increase in saxitoxin (a specific Na+ channel ligand) binding sites in immature rat brains after being subjected to anoxia. Hypoxia, suggesting that Na channels can regulate Na+ channels in excitable tissues. In the present study, we asked whether Na+ channel mRNA is regulated by O2 availability in excitable tissues of the developing rat. Newborn rats (postnatal 0-3 day) were exposed to low O2 environment (5±0.5%) for 27-30 days and then the brain, skeletal muscle and diaphragm were quickly removed for RNA extraction (m=9). Control litters were maintained in room air and studied simultaneously with the experimental rats. For the Northern blots, 20 µg of RNA was loaded in each lane on 1% agarose/formaldehyde gel. For the slot blots, serial concentrations (0.5-10 µg) of RNA were spotted in each slot. The CDNA probe is a 307 bp piece of Na+ channel IIα-subunit CDNA (from 4836 to 5142 bp) and is common all Na+ channels (I, II, III, III). Northern blots show a clear band with a size of ~4 kb. Quantitation of slot blots shows that Na+ channel mRNA markedly increased in brain (~80%) and skeletal muscle (~100%) in experimental rats; in contrast, no increase in mRNA was found in diaphragm with hypoxia. We conclude that long term hypoxia modulates Na+ channel mRNA differently in various excitable tissues of developing rats (Supported by NIH grant P01 HD32573, HL 39924, NS 32578, and a UCP grant).
SODIUM CHANNELS I


Little is known about the stimuli or mechanisms for regulation of Na channel mRNA in neurons. Physiological signaling pathways which may alter transcription of Na channel mRNA are being identified using cultured N1E-115 neuroblastoma cells as a model system. Messenger RNA is isolated and measured using an assay in which reverse transcription is followed by a competitive polymerase chain reaction. Previously we have reported that Na channel membrane current and mRNA are down-regulated in differentiated neuroblastoma cells grown in a Ca sequestrant (La3+) or nicotine (Quaide, Hirsh, and Sievert, Neuronci. Abs. 20:1506, 1994). This effect was selective since Kv 3.1 was not reduced under this condition. The result suggests that Ca alters transcription or lifetime of Na channel mRNA. The effects of other agents are currently being studied. Growth of cells in either nitrendipine, BAY K 8644, or depolarization with high K in the presence of valinomycin did not result in changes in Na channel mRNA abundance. Open Ca channels therefore appear to contribute little to regulation of mRNA under the normal growth condition. Addition of 40 μM veratridine to the culture medium for 3 days reduced Na channel mRNA by 80%. The action of veratridine on mRNA may be mediated by a putative internal Ca channel activation of Ca channels after depolarization, or more likely following a decrease in Ca influx through Na/Ca exchange. Supported by the National Multiple Sclerosis Society.

IMMUNOCHEMICAL AND ELECTROPHYSIOLOGICAL MEASUREMENTS OF SODIUM CHANNEL DISTRIBUTION ON UNINNERVED SKELETAL MUSCLES. B.D. Roberts and W.M. Roberts*, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Vertebrate neuromuscular transmission depends, in part, on high densities of acetylcholine receptors (AChRs) and voltage-gated sodium (Na) channels at the endplate. Much is known about the localization of AChRs to the endplate, but much less is known about Na* channels. To begin to understand how the specialized distribution of Na* channels on a muscle cell is maintained and is regulated, we have used immunofluorescent and electrophysiological methods to study the distribution of Na* channels on uninervated chick skeletal muscle myotubes growing in cell culture. We labeled myotubes with an antibody purified polyclonal antibody directed against the putative inactivation loop of the Na* channel, and then visualized the primary antibody distribution with a biotinylated secondary antibody and an avidin-conjugated fluorescent. Control myotubes were processed in parallel with experimental myotubes but without addition of primary antibody. The distribution of antibody labeling was examined by comparing blindly chosen areas of experimental and control myotubes. Most areas on experimental myotubes showed no significant label over control. However, 18% (20/112) experimental areas showed label intensity greater than 2 standard deviations above the control mean. Furthermore, only 18% (156/52) of the patches containing Na* channel showed significant labeling.

By using a tight seal whole cell electrode to record currents locally elicited by a loose-seal patch electrode, we were able to record currents from 23 patches of membrane separated by 10-100μm from different myotubes. For all but one patch, peak Na* current for each patch on a cell was between 0.5 and 1.5 of the mean for all patches from that cell. These results suggest that 1, Na* channels are distributed non-uniformly over uninervated myotubes but that the distribution does not have a sharp boundary, and 2, Nuclei within a single cell may differentially express unequal amounts of Na* channel.

DIFFERENTIAL DISTRIBUTION OF HUMAN BRAIN SODIUM CHANNEL SUBTYPE III mRNA: ANALYSIS BY LIGAND DETECTION REACTION (LDR). C.N. Lin* and G.B. Brown, Psychiatry and Behavioral Neurology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

In a previous study a reverse transcriptase-polymerase chain reaction (RT-PCR) coupled ligand detection reaction (LDR) assay was used to detect the differential distribution of human brain sodium channel (HBSC) subtype II and II mRNA by quantifying the relative amounts of subtype I to II radio-labeled LDR id products for 24.8 h, respectively. Although a higher proportion of "neural" myotubes were cultured in 0 mM Ca2+ (the recording solution which was spritzed onto the cells still contained Ca2+), Na* density was significantly reduced (9.5 ± 2.32 μA/pF; n = 11, p < 0.03), although the proportion of cells containing Na* was not altered (45%, 5/11).

Therefore, the early expression of Na* in developing myotubes appears to be a cell autonomous mechanism, possibly triggered by "muscle-derived factors" and involving Ca2+ entry. Nerve, however, may play a role in maintaining an accelerated level of Na* (Corfas & Fischbach, 1993:Neurosci. 13:2118).


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APPEARANCE OF SODIUM CURRENTS IN XENOPUS EMBRYONIC MYOCECTES MAY BE INDEPENDENT OF INNERVATION. E. Fribbebaru and A.L. Szpirer*. Dept. of Pharmacology, Univ. of Birmingham, Birmingham, B15 2TT. UK.

Embryonic Xenopus myocytes are functionally innervated and able to generate sodium-dependent action potentials in vivo within 12 hr from Stage 15 (when morphological and functional nerve-muscle begins to form). In this study, we examined the effect of innervation on the neuronal Na* current in a neonatal rat muscle, sodium current (INa) expression is regulated by innervation. Therefore, by comparing the expression of INa in myocytes isolated either at St. 15 ("anureal") or at St. 24 soon after they had been innervated ("neural"), we were able to assess the role of nerve in this aspect of electrical differentiation. The timing of the whole-cell patch clamp recordings (relative to St. 15) from "anureal" and "neural" myocytes was equivalent 24.8 ± 0.5 hr (n = 17) and 20.8 ± 2.6 hr (n = 18), respectively. Although a higher proportion of "neural" myocytes expressed INa (61% (11/18) vs 41% (7/17)), its density was not significantly different: 62.1 ± 23.3 μA/pF ("neural", n = 18); 25.1 ± 8.2 μA/pF ("anureal", n = 17; p > 0.2). The expression of INa was found to be dependent on extracellular Ca2+; however, when "anureal" myocytes were cultured in 0 mM Ca2+ (the recording solution which was spritzed onto the cells still contained Ca2+), Na* density was significantly reduced (9.5 ± 2.3 μA/pF; n = 11, p < 0.03), although the proportion of cells containing Na* was not altered (45%, 5/11).
714.11 Expression of Na channel 6 alpha subunit mRNA in the developing rat central nervous system. K.L. Schaller and J.H. Caldwell* Dept. Cellular and Structural Biology, Univ. CO Health Sciences Center, Denver, CO 80262

A novel sodium channel isoform (NaCh 6) has been isolated and characterized from rat brain (Schaller et al, J. Neurosci., 1995). Previous studies showed that transcripts for NaCh 6 are abundant in the adult rat brain and by in situ hybridization are expressed in both neurons and glia. Here the regional distribution of NaCh 6 mRNA expression in the developing rat brain was investigated by in situ hybridization. At embryonic days 15 and 18 weak hybridization is detectable over developing neuroepithelium. Transcript levels increase during postnatal development throughout the brain and spinal cord. By PINaCh 6 mRNA is detected in the pyramidal cells and dentate gyri of the hippocampus, the granule cells of the cerebellum, and throughout the olfactory bulb. In these regions the expression of this mRNA becomes progressively more intense with maturation. In the CNS, NaCh 6 expression is highest at P7-P14 and then falls with further maturation. Expression of NaCh 6 transcripts is seen beginning at P21 in both the brain and spinal cord. Thus it appears that the expression of NaCh 6 is both temporally and regionally regulated in the developing CNS.


Rat brain sodium channels consist of a 6-subunit, a 7-subunit and an a-subunit. Currenty there are at least 4 different a-subunits (forms I, II (Noda et al., Nature 320:188), III (Kawano et al., FEBS Lett 223:417) and a putative glial sodium (NaG; Guarrero et al., PNAS 89:7273)). During late embryonic-early postnatal development of the rat nervous system, the expression pattern of sodium channel subunits is altered: expression of form I and the 6-subunit increases, and form III decreases. The adult expression pattern is attained at approximately 3 weeks postnatal age. Thus the developmental progression of sodium channel subunit expression is coincident with the period of most active myelination in the CNS. It is not known, however, if this progression is dependent upon myelination. We have therefore compared sodium channel 6a- and 6b-subunit mRNA expression in myelin deficient (md) and unaffected male littermates (20-22 days postnatal age). md is a sex-linked mutant of Wistar rats characterized by a near total lack of myelin in the CNS. Subunit expression was studied in the hippocampus, cerebellum, spinal cord and dorsal root ganglia (DRG) using in situ hybridization with digoxigenin labeled riboprobes. The expression pattern we observed in unaffected animals was generally in accord with previous studies on adult animals. In the 3 CNS regions, the intensity of subunit expression was: II>>III>III with no detected expression of NaG. In DRG neurons, the 6a- and 6b-subunits were prominently expressed, with less expression of 6b-subunit III. DRG neurons did express NaG mRNA. In md rats, the pattern of expression of sodium channel a-subunits I, II, III and NaG, and the 6b-subunit, was indistinguishable from the pattern observed in unaffected littersates in all tissues studied. This similarity indicates that CNS myelination is not necessary for progression from the embryonic to the adult sodium channel subunit expression pattern.

715.1 MODELLING IMPLIES A SPECIFIC SODIUM CHANNEL TRANSITION DEFECT IN EPIDEMIC PERIODIC PARALYSIS. W.B. Hansaoon & R.J. Tsushima. *Biophysics Group, Dept. Med., Univ. Guelph, Guelph, GNT, N1G 2W1, Canada; Cardiology, Dept. Med., Univ Toronto School of Med. & CCRW, The Toronto Hospital, 100 College St, Toronto, GNT, M5G 2A6, Canada.

Equine hyperkalemic periodic paralysis (EHPP) is associated with a skeletal muscle sodium channel a-subunit mutation corresponding to Phet1412Leu in the rat 6a sodium channel (Rudolph et al., Nature Genetics 1:244, 1992). Current from these channels containing this mutation differ from the wild-type in several ways: (1) whole-cell peak currents decay several-fold more slowly and decay incompletely, producing a pedestal of sodium current; (2) whole-cell peak currents recover from inactivation approx. one order of magnitude more rapidly; (3) the steady-state inactivation curve is shifted to the right with no change in slope; (4) single channel currents produce brief openings during prolonged depolarizations; (5) mean single-channel open-time is increased by approx. 40-90%. Action parameters are not affected.

The phenotypic differences between control and EHPP sodium channels are predicted by moderate changes in rate constants (in the sodium channel gating model developed by Kuo and Bean [Neuron 12:819, 1994]) involving transitions between the open ("O") and open-inactivated ("I") states. Reductions of O\textsubscript{up} combined with increases of O\textsubscript{up}, each of one order of magnitude or less, in the Kuo/Bean model reproduce precisely the whole-cell inactivation gating abnormalities observed with the equine EHPP mutation. Monte-Carlo simulations also predict the single-channel experimental results. Alterations of rate constants representing other molecular transitions in this model do not predict the complete EHPP phenotype. These results strongly suggest that S3-DIV is critical for normal binding of the inactivation gate with its receptor.

 Supported by MRC Canada (WJHH) and MDA Canada (PHB and RGT).

715.2 CYSTEINE MAPPING IN THE SELECTIVITY REGION OF THE SODIUM CHANNEL. S.-F Chen*, H.A. Hartmann* and G.B. Kirsche*. Depts. of Molecular Physiology and Biophysics and Anesthesiology, Baylor College of Medicine, Houston, TX 77030.

The pharmacology of brain and skeletal muscle sodium channels (NaChs) is different from that of the cardiac isofrom, but the ionic selectivity is the same in these different types of NaChs. A conserved lysine residue in the SS2 segment of domain III is critical for selectivity in NaChs (Heinemann et al., 1992; Nature 356:441-443). A single point mutation in a cysteine residue at 322 of the domain III of NaCh switches the pharmacology to the type of brain/skeletal muscle NaChs (Satin et al., 1992; Science 256:2200-2203). A combined approach by systematic cysteine substitution and sulhydryl modifying compounds was carried out in the S22 of domains III and IV of the switched NaCh to map the residues which may line the narrowest part of the pore.

The sulhydryl modifier Antimycin A, cysteamine, capsaicin and N-ethylmaleimide (NEM) inhibit the conductance of D1713C and decreased the conductance of W1712C, M1421C, and W1430C. These results indicate that the SS2 of domain III and IV are not symmetrically aligned. D1713 is at external vestibule while the other three are already inside the narrow region. MTS cannot modify K1418C and A1710C. This suggests that these two residues are deep inside the pore where anions cannot reach. The positively charged 2-aminoethyl methanethiosulfonate (MTSEA) can still modify K1418C and A1710C with very slow kinetics. This indicates that the large molecule cannot freely rotates in the vicinity of these two residues. Selectivity amongst alkali metal ions was drastically reduced in the K1418C mutant such that Ra+/K+ favors 1.2, versus 0.1 in control. The sequence of selectivity for all the other mutants remained as the Eisenman sequence X or X. These results suggest that the side group of K1418 contributes to much of the spatial restriction at NaCh selectivity filter.
T15.3 TTX DIFFERENTIALLY MODULATES FAST INACTIVATION GATES IN ADRENA L CHROMAFFIN CELL Na+ CHANNELS. F. Hamburger & R. J. Solomon,* Dept. of Mol. & Cell. Pharmacology, University of Miami, Miami, FL 33101

Na+ current inactivation in chromaffin cells can be described by a kinetic model that includes two rate-limiting fast inactivation states (\(I_{\text{fast}}\) and \(I_{\text{slow}}\)) that differ primarily in their time course of recovery from inactivation (\(t_{\text{fast}} = 8 \, \text{ms}, \, t_{\text{slow}} = 140 \, \text{ms} \equiv 80 \, \text{mV})\). \(I_{\text{fast}}\) and \(I_{\text{slow}}\) interact in a strict first-order manner such that inactivated states do not occur unless channels first recover from inactivation. Repetitive depolarizations (5 ms \(\pm 20 \, \text{mV}\)) at 5 ms intervals increase the fraction of channels in \(I_{\text{slow}}\), since many channels recover from \(I_{\text{fast}}\) during a 5 ms interval but few recover from \(I_{\text{slow}}\). Measurements of cell membrane capacitance (\(C_m\)) can be used to monitor the fraction of channels in \(I_{\text{slow}}\). (K. I. Ruff & R. J. Solomon, 1994 Neuron, 12.) A 5ms depolarization (+20mV) produces a transient increase in \(C_m\) as Na channels inactivate and then recover from inactivation. Inactivation of these channels in \(I_{\text{slow}}\) is significant to 1 mV phase. Thus TTX binding appears to retard recovery from \(I_{\text{slow}}\) significantly without altering the number of channels that enter \(I_{\text{slow}}\) during a 5ms depolarization. In contrast, recovery from \(I_{\text{fast}}\) is not modulated by TTX since the repetitive pulsing paradigm still produces an increase in \(C_m\) amplitude. Thus TTX and related toxins may provide important tools for distinguishing the molecular mechanisms underlying these two forms of fast inactivation.


It has been hypothesized that slow inactivation must be compromised by mutations causing channelopathies (e.g., periodic paralytic (PP)) to account for the duration of the paralysis observed in patients with HYP (R. Ruff, Biophys. J., 66:26, 1994). We are studying a mutation (T764M) in the rat skeletal muscle sodium channel that corresponds to one of the human HYP mutations (T704M) and have compared slow inactivation of wild type and T764M channels stably expressed in HEK293 cells.

Wild type channels exhibit slow inactivation in HEK293 cells. Slow inactivation develops with a similar time course to that observed for sodium currents in rat skeletal muscle. With the use of the Na+ channel blocker neomycin, the persistent sodium current was reduced to 30% of control. However, when the fast sodium current is reduced by 30%, the slow sodium current accounts for 40% of control current. This indicates that slow inactivation is indeed effective in HYP mutant sodium channels and may contribute to the maintenance of paralysis in patients with HYP.


One previous study explained the two contradictory evidences on the mechanism of invertebrate phototransduction, the process of PIP2 hydrolysis breakdown into IP3 and diacylglycerol (DG) is necessary for the generation of photoreceptor whereas the product, IP3, is not required. We hypothesized that the local charge movement caused by PIP2 hydrolysis near the inner surface of rhodopsin membrane controls the gating of the non-voltage sodium channel. The hydrolytic charge liberation was confirmed on the suppression of photoreceptor not only by the injection of charge chelators such as neomycin, spermine but stoppage of PI turnover by IEIMX, L34 and R5022. We postulated that the light sensitive sodium channel is activated in the charge sensitive manner. The indirect increase of IP3 inside the type B photoreceptor by perfusing NLC3 containing ASW augmented the initial component of photoreceptor by 20% increase in amplitude. On the other hand decrease in the injection of acetate buffer(pH 4.6) maximally suppressed the photoreceptor by 70% then return to normal within 20 min. The size of photoreceptor was found to be proportional to the dissociation of phosphoryl which was obtained by 3P-SNP experiment. Here we assume that phosphatase C, hydrolysing PIP2, may be activated rapidly by light in retinas synaptic. Since the voltage-dependent sodium channel in the squid axon has been known to be inactivated to the internal pH change, acidic/basic, it is likely that the B-photoreceptor is activated by light in the membrane charge dependent fashion.


Voltage clamp techniques based on whole cell patch recording were used to study transient voltage-activated currents of frog tectal neurones. The classical preparation at room temperature. The external solution contained oxygenated and buffered saline solution (100 mM NaCl). The pipette solution usually contained Cs sulphate (sometimes replaced by K gluconate) plus ATP, EGTA and 5-10 mM Na+. In forty neurones held at 50 mV depolarizing voltage steps excited a fast inward sodium current with threshold at about -40 mV and peak at -1 ms. Under these conditions, sodium currents were always followed by a large transient outward current carried in approximately equal degree by Cs or K ions with peak at about 3.5 ms. The same type of fast inward sodium current was also activated by command potentials more negative than -90 mV but in this case it was not followed by any outward component corresponding to the Cs current observed at depolarized potentials. Extrapolated TTX (1 μM), ouabain (50 μM) or Cs (10 mM) simultaneously blocked inward and outward current induced by depolarizing command steps and also blocked the isolated sodium current evoked by voltage steps negative to -90 mV. The present data suggest that frog tectal neurones possess a novel type of sodium activated transient cationic current characterized by a rapid change in the selectivity of the sodium channel from sodium to potassium/celium following the initial entry of sodium into the channel itself. This current possesses some similarity to the sodium/potassium pump current of peripheral cells but it seems to present the unusual properties of rapid kinetics and voltage-dependent activation.
DIFFERENTIAL PROPERTIES OF VOLTAGE-DEPENDENT Na+ CHANNELS FOLLOWING AXOTOMY IN CUTANEOUS AFFERENT DRG NEURONS OF ADULT RAT. M.A. Bizzi, S.G. Warnes*, J.D. Kocsis, Dept. of Neurology, Yale School of Medicine, New Haven, CT 06510 and Neuroscience Research Center, VAMC, West Haven, CT 06516.

Studies of the behavior of Na+ currents particular to cutaneous afferent dorsal root ganglion (DRG) neurons following chronic axonal injury should increase our understanding of the hypersensitivity of these neurons following chronic axonal injury. Cutaneous afferent DRG neurons, in short-term culture, were identified by immunofluorescence labelling of the sciatic nerve with Fluoro-gold. Using the whole-cell and bled-patch-clamp recording techniques, Na+ currents were recorded from these neurons pre- and post-axotomy and tested for sensitivity to 1 mM TTX and 100-400 μM lidocaine. Na+ currents in neurons post-axotomy, in contrast to controls, tended to be blocked by TTX, and by relatively higher concentrations of lidocaine. Kinetically slow, TTX-resistant Na+ currents predominated in control neurons. Predominantly kinetically fast, TTX-sensitive Na+ currents emerge post-axonotomy. Steady-state activation and inactivation curves constructed from Na+ current recordings of semicircular membrane biops (fragments of membrane 3-4 μm diameter corresponding to 0.3-0.7 μm) excised from cutaneous afferent DRG neurons pre- and post-axotomy revealed that conductances post-axonotomy had a decreased slope factor, broader window currents, and splay of steady-state inactivation in comparison either to slow Na+ conductances in control neurons, or to kinetically equivalent (fast) counterparts in non-axonotomized, non-cutaneous afferent DRG neurons. These findings suggest that, following axotomy, new subpopulations of Na+ channels occur in cutaneous afferent DRG neurons. This may confer altered neuronal excitability, contribute to ectopic impulsive generation, and under pain and paresthesias following chronic axonal injury.

SODIUM CHANNELS III

MUSCARIC MODULATION OF SODIUM CURRENT BY ACTIVATION OF PKC IN RAT HIPPOCAMPAL NEURONS. A.R. Cantrell*, T. Schafer, and W.A. Catterall, Department of Pharmacology, University of Washington, Seattle, WA 98195.

Voltage-gated sodium channels play an important role in neuronal excitability in the hippocampus but are infrequently recognized as targets for neuromodulation. Phosphorylation of brain sodium channels by protein kinase C (PKC) decreases peak current and slows time-independent inactivation. Hippocampal muscarinic receptor activation activates PKC. Therefore, we have tested whether sodium channels can be modulated by muscarinic receptor activation in acute, isolated adult rat somatic hippocampal neurons using whole-cell voltage-clamp recording.

Application of the muscarinic agonist, carbachol 10μM, reduced peak current an average of 29.7% in 28/29 cells tested and slowed macroscopic inactivation at all potentials. No change in the voltage dependence of activation or inactivation was observed. These effects were modulated via PKC as they were eliminated when a specific PKC inhibitor peptide (PKCII-31) was included in the pipette solution and mimicked by the extracellular activation of the PKC activator, OA.

These results demonstrate that muscarinic activation of endogenous receptors on hippocampal neurons strongly modulates sodium channel activity by activation of PKC. Such modulation is expected to have potent effects on neuronal activity.


We examined whether protein kinase C (PKC) is involved in the regulation of Na+ channels in cultured bovine adrenal chromaffin cells. The chromaffin cells contained PKC α, γ and δ isoforms, but not β, η and ζ isoforms. The immunoreactive PKC α and δ but not α were translated from cytosol to membrane by a 15 h treatment of the cells with 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 nM) and phorbol 12, 13-dibutyrate (PDBu; 100 nM), activators of PKC. The treatment decreased the Bmax of [3H]saxitoxin binding to the cells without altering the Kd value, and also reduced the veratridine-induced [3H]saxitoxin influx, while 4α-TPA, an inactive analog, had no effect. The effect of TPA was abolished by H-7, an inhibitor of PKC. Even a 2 h pulse-treatment with PDBu followed by a 13 h incubation without PDBu caused the comparable decrease in [3H]saxitoxin binding with the continuous 15 h treatment.

These results suggest that functional Na+ channels are down-regulated by a delayed-effect of short-term activation of PKC α and/or δ isoforms.
716.3 MODULATION BY INTRACELLULAR FATTY ACIDS OF HUMAN MUSCLE SODIUM CHANNELS EXPRESSED IN HEK293 CELLS IS DEPENDENT ON A SUBUNIT DISORDER AND PATHS OF DELIVERY. S. J. Metcalfe and J. E. Bieger.* Deps. of Pharmacology and Neurobiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and John Hopkins Applied Research Corp., Bayamon, PR. 00959.

Free fatty acids (FFA) participate in signalling pathways, including the arachidonic acid (AA) cascade and the eicosanoid cascade. These lipids are implicated in the direct and indirect modulation of a spectrum of voltage-gated ion channels. Skeletal muscle Na+ channels can be either activated or inhibited by FFA exposure; the response is dependent on both FFA structure and site of exposure. For example, DM OA augmented the voltage-activated Na+ current of recombinant SKM1 skeletal muscle Na+ channels expressed in Xenopus oocytes. (IC50 = 13.0 and 3.6 pM) over a 20 min period. Similar results were seen with 5 μM oleic acid (OA). Na+ currents in cells expressing H1 cardiac muscle Na+ channels were insensitive to AA treatment (Figure 1) and exposure to OA inhibited the H1 currents over a 20 min period by 29% (13.5 ± 5% SE at 5). Increase in SKM1 current was not accompanied by shifts in voltage dependence of activation, steady-state inactivation or markedly altered kinetics of inactivation of the macroscopic current. Fatty acids increased Na+ permeability and decreased the activation and inactivation times of Na+ channels.

716.5 MODULATION OF SODIUM CHANNELS BY INSECT-SELECTIVE SCORPION AND SPIDER TOXINS. T. M. Norris, D. A. Adams and H. F. Adams. Deps. of Entomology and Neuroscience, University of California, Riverside, CA 92521.

A subset of arthropod Na+ channel toxins are known to be selective for insect neurons. Two insect-selective toxins which show different types of Na+ channel modulation are LqhTII and μ-Aga-IV. Both toxins cause repetitive activity in insect motorneurons. LqhTII increases both the amplitude and duration of evoked synaptic currents at the neuromuscular junction. In contrast, μ-Aga-IV produces a dramatic increase in spontaneous transmitter release. Whole-cell patch clamp recordings were performed to examine the effects of these toxins directly on sodium currents in insect neurons (Helobius virens). LqhTII (200 nM) slows the rate of Na+ channel inactivation and more than doubles peak current, effects which are consistent with an α-channel-activating action. Mammalian neurons (rat DRG) were markedly less sensitive to this toxin. μ-Aga-IV dramatically shifts the voltage dependence of activation and inactivation of Na+ channel activation in insect neurons to more negative potentials and slows the rate of channel inactivation. These effects resemble in some respects the action of mammalian α-channel toxins. μ-Aga-IV does not produce any toxicity upon intracranial injection into mice and had no significant effect on rats' Na+ neuronal sodium currents. These data demonstrate that LqhTII and μ- Aga-IV selectively modulate insect sodium channels in ways that explain their paralytic neuromuscular actions, and that the modulatory actions of these toxins resemble those of the mammalian α- and β-channel toxins, respectively.

716.7 EXTRACELLULAR APPLICATION OF QX-314 BLOCKS SODIUM CHANNELS AND CAUSES LOCAL ANESTHESIA. K. B. Rietsch, M. Khatibzadeh, D. R. Bridges, L. Lee and J. C. Hunter. Deps. of Anesthesiology, Hoechst Marion Roussel, Palo Alto, CA 94306. QX-314, the quaternary derivative of lidocaine, is widely regarded to block Na+ currents only following intracellular application, as its permanent positive charge is thought to restrict lipid bilayer permeation. Consequently, QX-314 and other permanently charged Na+ channel blockers are not believed to be local anesthetics (see Hille, 1992, p. 405). To address this assumption, we have examined the ability of QX-314 to block action potential propagation and cause local anesthesia. Reduction of sodium current conduction is proportional to the degree of Na+ channel blockade. Accordingly, extracellular recordings were made of C-fiber component action potentials in rat vagus nerve in vitro. Cumulative concentration-inhibition curves to QX-314 were constructed using 10 min incubations to 1, 10, 100 and 1000 μM compound. Tonic block occurred at a slow rate of nerve stimulation (0.03 Hz), and phasic block with a 30 Hz burst of nerve stimulation at the end of each 10 min period. High frequency nerve peristimuli shifted the IC50 for lidocaine only marginally, from 220 μM (tonic) to 210 μM (phasic). In contrast, the QX-314 IC50 were >1000 μM (tonic) and 380 μM (phasic). Frequency-inhibition curves (0.1-30 Hz) were measured in the presence of -IC50s for tonic block. Lidocaine, a kinetically fast Na+ channel blocker (t1/2 0.3 sec in the heart), produced appreciable phasic block only at 30 Hz, whereas QX-314 (t1/2 6 sec) caused significant phasic block even at 0.1 Hz. To measure local anesthesia, interscalenial injections of varying concentrations of QX-314 were made on the lower back of guinea-pigs. At 5, 10, 20 and 30 min after dosing, skin sensitivities were tested by prick the skin with a needle. The number of negative responses (no redness, no pain) at different IC50 values (expressed in %) of 16 for lidocaine and 0.235 for QX-314. The onset and duration of local anesthetic effects produced by QX-314 were application of QX-314.


In this work we used primary cultures of chick embryo sympathetic neurons as a model system to study the mechanisms underlying the actions of known and novel toxins on voltage sensitive sodium channels. Pharmacologically isolated inward sodium currents (INa) elicited by depolarizing stimuli were examined using the whole-cell technique in cultures after 48 hrs incubation. INa was described in terms of ionic nature, voltage dependence of activation and inactivation, and sensitivity to specific toxins. Tetrodotoxin and mu-opioid receptor agonists blocked INa to nearly 40% and 20%, respectively. Voltage sensors of the sodium L, Q, and H, heerex neurons inhibited inactivation at the -95 mV range and caused a delay in this process as well as currents blockage at this level. The tetrodotoxin type 1 (PTX-1) from P. brevis shifted toward more negative potentials the voltage dependent processes and delayed INa reactivation. In other experiments, the actions of aequorin were monitored in the presence of aequorin, Dibromocin, displaces -Haxatone in rat brain synaptosomes, and as expected for a site 1 toxin, decreased by nearly 40% INa amplitude. It also shifted toward more negative potentials the voltage dependence of inactivation. A toxic extract from the dionidogale G. leucurris, did not affect -H-PTX3 binding, decreased INa amplitude by 20% and shifted by 20 mV, respectively. Different from the dionidogale G. leucurris reactivation were not altered by this dionidogale extract. (Supported by GM80102 and NH81910)
716.9

The alkaid toxins veratride and batrachotoxin bind to a site (on the so-called II) on the voltage dependent sodium channel where they cause persistent activation of the channel and depolarize the cell. Tetrodotoxin and saxitoxin bind to a different site on the sodium channel and can block the effects of veratride and batrachotoxin. Other known modulators of sodium channels include anticoagulants and local anesthetics which inhibit sodium currents and displace $[^{3}H]$batrachotoxin binding. Besipirine (H 749) is a compound undergoing clinical trials for efficacy in treating Alzheimer's Disease. Among other pharmacological effects, besipirine inhibits voltage dependent sodium and potassium channels. We present here a more detailed study on the interaction of besipirine with voltage dependent sodium channels. Besipirine displaced $[^{3}H]$batrachotoxin binding with an IC50 of 5.5±0.2 μM in rat brain vascular preparation and inhibited veratride-stimulated increases in intracellular free sodium (Na+) and calcium (Ca++) in primary cultured rat cortical neurons in a concentration dependent manner. Furthermore, besipirine dose-dependently inhibited veratride-stimulated release of [3H]norepinephrine (NE) from rat cortical slices. When examined in greater detail, besipirine was found to inhibit $[^{3}H]$batrachotoxin binding in vivo, blood and membranes competitively. However, when examined in rat brain synaptosomes, the antagonism by besipirine was found to be non-competitive. BESIPRIPINE has been characterized as the mixed potentiation of [Ca++]1 induced by veratride decreased with increasing concentration of besipirine. These results are discussed in relation to possible anticonvulsant activity of besipirine.

716.10

Pyrethroids make up a large class of potent neurotransmitter insecticides whose principal target is the voltage-gated sodium channel. In intact nerves, pyrethroids induce prolonged sodium currents and afterpotentials which lead to uncontrolled repetitive firing or use-dependent nerve block. To further explore the molecular basis of pyrethroid action on sodium channels we employed the heterologous expression of sodium channel subunits in Xenopus oocytes. The rat brain Ila sodium channel α subunit and the rat brain β2 subunit mRNAs were synthesized in vitro from cloned cDNAs. The α subunit mRNA was injected into oocytes either alone or in combination with the β2 subunit mRNA and the actions of the pyrethroid deltamethrin on expressed sodium currents were assessed by two-electrode voltage clamp. Deltamethrin prolonged the time course of sodium channel inactivation and produced tail currents following repolarization that were qualitatively similar to the effects produced on sodium channels in intact nerves. The presence of the β2 subunit enhanced rapid inactivation, as anticipated, but did not modify the effects of deltamethrin. These findings imply that the binding domain involved in the neurotoxic action of pyrethroids is associated with the sodium channel α subunit. Other pyrethroids also prolonged sodium channel inactivation and produced sodium tail currents in this assay system. The rate of tail current decay in this system was found to be compound-dependent.

716.11

In the brain, the number and types of VOSC change1 and their ion-specific affinity increases during development. We are investigating whether other properties of VOSC undergo changes during forebrain development. Sodium channel activity from newborn (P0) and postnatal day 15 (P15) rat brains were studied in planar lipid bilayers in the presence of BTX and in symmetrical 200 mM NaCl. The steady state activation curve showed a hyperbola with a maximum amplitude. The midpoint potential values were -42mV and -47mV for channels derived from P0 and P15 forebrains respectively. The maximal fractional open time was lower in channels derived from P0 than P15 due to a higher proportion of short- and long-lived channel closures in channels from P0. The level of Li-induced open channel block did not change during development. Li blocked the open state of brain channels with about ten times lower affinity than that of muscle derived channels2. The higher affinity of brain channels was mainly due to a lower k∞ value, indicating that the conformation of the channel structures defined. The Li binding site is not identical in brain and muscle-derived sodium channels; while they appear to be the same in the various brain sodium channels. Sheneman et al., 1985; Villegas et al., 1994; Zamponi et al., 1993.

716.13
S-CONOTOXINS, A FAMILY OF SUBTYPE-SPECIFIC CONUS PEPTIDES WHICH INHIBIT INACTIVATION OF VOLTAGE-SENSITIVE SODIUM CHANNELS. K. Shom,1 M. M. Grilli,2 H. Tatsumi,2 W. Ribbe3, W. B. Gray1, J. S. Imperial1 and D. M. Olivera.1 Dept. of Biology,1 Univ. of Utah, Salt Lake City, UT 84112; Max-Planck-Institut fuer experimentelle Medizin,2 D-37075 Gottingen, Germany; and3 Insitut fur Toxikologische Chemie der Universitat, D-80333 Munich, Germany.

A vertebrate-specific δ-conotoxin, δ-PV1a (the "lock-jaw" peptide) was purified from the venom of the conus snail Conus ventosa. The venom-hunting cone snail (Shom et al., 1995, Biochemistry, 34, 4913). The two δ- conotoxins previously characterized, δ-TXVa (the King-Kong peptide) and δ- Gm11 (Hillery et al., 1989, Biochemistry, 28, 356; Shom et al. Biochemistry, 1994, 33, 11420) were from mollusc-hunting cone snail venom, and exhibited potent inhibition of mollusc-voltage sensitive sodium channels. Like the other δ-conotoxins, δ-PV1a is a very hydrophobic peptide, with 29 amino acids, and 3 disulfide bridges. Although the disulfide bridge framework is conserved in all three δ-conotoxins, the non-cysteine amino acids are not conserved in the three family members. δ-PV1a elicits excitatory responses in molluscs, but is inactive in mammals, even at doses 100 fold higher. The peptide inhibits sodium channel inactivation in both cloned mammalian sodium channels expressed in Xenopus oocytes, as well as in cultured mammalian neurons. The δ-conotoxins are a promising group of Conus peptides for discriminating between various vertebrate subtypes of voltage-sensitive sodium channels. δ-PV1a is generally inactive in vertebrate systems, δ-conotoxin Gm11 causes excitatory symptoms but only in immature mammals. In contrast, δ-PV1a exhibits broad activity in a wide range of mammalian tissues for all three δ-conotoxins and for sequences that have been characterized, this makes it possible to systematically identify additional δ-conotoxins from Conus species using molecular biological techniques.

716.14
NEW CONOTOXINS WHICH BLOCK NEURAL SODIUM CHANNELS. J.M. McIntosh,1 A. Hassoun,2 M.E. Spir,3 W.R. Gray,1 W. Li,1 M. Marsh,1 D.R. Hillyard,1 H. Tatsumi,2 W. Stuhmer,2 and B.M. Olivera.1 Dept. of Biology,1 Pathology2 and Psychiatry3, Univ. of Utah, Salt Lake City, Utah, 84112; Dept. of Neuroscience,4 Inst. of Life Sciences, Hebrew Univ. of Jerusalem, Jerusalem, Israel; and Max-Planck-Institut fuer experimentelle Medizin,5 D-37075 Gottingen, Germany.

We previously characterized ω-conotoxins, µ-conotoxins and δ-conotoxins, which target voltage-sensitive calcium channels, voltage-sensitive sodium channels and inhibit sodium channel inactivation, respectively. We report a new family of peptides, the ω-conotoxins, which target voltage-sensitive sodium channels. The 3 ω-conotoxin peptides, ω-conotoxins MrV2a/b, were purified from the venom of Conus marmoreus. Their structures were confirmed by mass spectrometry, molecular cloning and sequencing. The 3 ω-conotoxin peptides, ω-conotoxins MrV2a/b, were purified from the venom of Conus marmoreus. Their structures were confirmed by mass spectrometry, molecular cloning and sequencing. The 3 ω-conotoxin peptides, ω-conotoxins MrV2a/b, were purified from the venom of Conus marmoreus. Their structures were confirmed by mass spectrometry, molecular cloning and sequencing. The 3 ω-conotoxin peptides, ω-conotoxins MrV2a/b, were purified from the venom of Conus marmoreus. Their structures were confirmed by mass spectrometry, molecular cloning and sequencing. The 3 ω-conotoxins are also active in vertebrate systems; unlike the µ-conotoxins which are specific for muscle Na channels in adult rats, ω-conotoxins potentially inhibit neuronal subtypes. Although the µ-conotoxins block voltage-sensitive sodium channels like the µ-conotoxins, the disulfide framework of the µ-conotoxins is that of the ω- and δ-conotoxins but not of the µ-conotoxins. Thus, together the ω-, µ- and δ-conotoxins define a superfamily of Conus peptides.
SODIUM CHANNELS IV


Voltage-sensitive Na⁺ channels are the membrane proteins responsible for the rising phase of the action potential observed in excitable cells. Na⁺ channels isolated from rat brain are a heterogeneous complex composed of an α subunit and two auxiliary β subunits, β1 and β2. β1 has been shown to be non-covalently associated with α while β2, has been shown to be by disulfide bonds. The β1 subunit has been cloned, sequenced and expressed and shown to play a modulatory role in sodium channel kinetics and expression levels. We now describe the primary structure of the rat brain Na⁺ channel β2 subunit. The deduced primary structure of β2 shows that it is a 20,000 dalton protein that contains a cleaved signal peptide, a single putative transmembrane domain, and 4 potential N-linked glycosylation sites. β1 and β2 subunits share topological similarities, yet are not homologous at the nucleotide or amino acid levels. Comparison of the β2 peptide sequence with other known sequences in the peptide database shows that β2 contains a domain that is homologous to a neuronal cell adhesion protein that has been implicated to play a role in the formation of the developing nervous system. Northern blot analysis of mRNA expression suggests that β2 is limited to the central nervous system. Coexpression of Type IIa α and β2 subunits in Xenopus oocytes results in a β2 concentration-dependent increase in peak current amplitude; an increase in the rate of macroscopic inactivation, and a small hyperpolarizing shift in the voltage-dependence of inactivation. These functional effects of β2 appear to be additive with β1. Thus, β2 subunits may function as important regulators of Na⁺ channel expression, modulating electrical excitability in the embryonic and adult central nervous systems.

117.7 GENES ENCODING THE β1 SUBUNIT OF VOLTAGE-DEPENDENT BRAIN SODIUM CHANNEL IN RAT, MOUSE AND HUMAN CONTAIN AN INTRON. Sylwana P. Dib-Hajj, and Stephen G. Waxman. Department of Neurology, Yale University School of Medicine, New Haven, CT and Neuroscience & Regeneration Research Center, PVA/VA, VA Medical Center, West Haven, CT 06516.

In an effort to understand the functional role of the different functional Na⁺ channel subtypes, we recently described a transcript (β1.2) that is closely related to the voltage-dependent brain sodium channel β1 transcript (β1.1). CDNA of the β1.2 transcript was amplified and subcloned in two neuronal β1.2+β1 subunit expressing systems which displayed a 'normal' current similar to that of neuronal β1.2 subunit; suggesting that the β1.2 subunit can complement the β1 subunit to form a functional channel. We have now used the β1.2 subunit as we have a functional expression of two functional β1.2 subunits, peak I_{Na} was observed over time in Xenopus oocytes heterologously expressing β1.2 alone or with β1 subunit. The maximal peak expressed in the β1.2 current occurred at least two times faster in the presence of the β1 subunit than when β1 was expressed alone. To test whether the β1 subunit can modulate the voltage-dependent properties of β1.2, we investigated the voltage dependence of the β1 subunit expressing neurons. In β1 subunit expressing neurons, Na⁺ channel injection was delayed by 24 or 48 hours. Regardless of subunit RNA injection order or the length of delay between injections (548 hours) all cells receiving β1.2 subunit RNA exhibited significantly greater I_{Na}/I_{Na} ratios than cells injected only with RNA encoding β1 subunit, "post-pункциing" β1 induced a significant decrease in slow-inactivating (mode 2) whole cell I_{Na} with no net change in channel density (as estimated from weighted whole cell peak I_{Na}) it seems likely that the observed shift in gating mode represents a conversion of channels already in the plasma membrane from mode 2 to mode 1 conformation.


Experiments were performed in a Chinese hamster ovary cell line that stably expresses the α1A subunit of the cardiac sodium channel (CNAI-1). Electrophysiological studies demonstrate that CNAI-1 cells generate a rapidly inactivating, inward sodium current with normal time course and voltage dependence (Sconier et al., 1990; Knap et al., 1993). The neurotoxin, veratridine, causes persistent activation of sodium channels at resting membrane potential by blocking inactivation and is blocked by scorpion venom. Veratridine-induced [14C]-guanidine flux in CNAI-1 cells is a dose dependent manner with maximal flux occurring at 125-300 μM. Scorpion toxin (6 μg/ml) potentiated this flux up to 14-fold. Veratridine-induced flux in the absence and presence of scorpion toxin (6 μg/ml) was linear with time up to at least 60 min and 45 min, respectively. TTX inhibited the veratridine (50 μM)-induced flux in the absence and presence of scorpion toxin (6 μg/ml) with an IC50 of 6.9 and 33.8 μM. Several anichologs, the sodium channel blocker, BW 100367 and a number of our putative soma channel blockers inhibited veratridine induced [14C]-guanidine flux as shown below:

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 ± S.E.M (μM)</th>
<th>IC50 ± S.E.M (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMIN-TRIKE</td>
<td>44.4 ± 1.4a, n=5</td>
<td>32.6 ± 0.9, n=2</td>
</tr>
<tr>
<td>ה</td>
<td>100 ± 6.9</td>
<td>44.4 ± 1.4</td>
</tr>
<tr>
<td>RUZELME</td>
<td>2.15 ± 0.96, n=4</td>
<td>1.05 ± 0.33, n=2</td>
</tr>
</tbody>
</table>

In conclusion, this flux assay in CNAI-1 cells is a useful high throughput screen for compounds with putative type IIa sodium channel blocking activity.

117.3 FUNCTIONAL EFFECTS OF NEURONAL Na⁺ CHANNELS SUBUNIT ON THE HUMAN SKIN. L. J. Potts & W. A. Apers. Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The mammalian adult skeletal muscle voltage-sensitive Na⁺ channel underlying the sodium currents observed in the mammalian skeletal muscle. Functional voltagesensitive Na⁺ channels can be heterologously expressed in Xenopus oocytes using RNA encoding the α-subunit alone but display anomalously slow inactivation when expressed under mammalian (encoding mode 2) unless coexpressed with neuronal β subunit RNA, suggesting a modulatory role for that auxiliary subunit (which is closely related to the neuronal skeletal muscle Na⁺ channel β-subunit). In order to investigate the role of the β-subunit in the expression of functional Na⁺ channels subunits, RNA encoding the functional Na⁺ channel subunits were injected into Xenopus oocytes. Na⁺ channel injection was delayed by 24 or 48 hours. Regardless of subunit RNA injection order or the length of delay between injections (548 hours) all cells receiving β1.2 subunit RNA exhibited significantly greater I_{Na}/I_{Na} ratios than cells injected only with RNA encoding β1 subunit, "post-injected" β1 induced a significant decrease in slow-inactivating (mode 2) whole cell I_{Na} with no net change in channel density (as estimated from weighted whole cell peak I_{Na}) it seems likely that the observed shift in gating mode represents a conversion of channels already in the plasma membrane from mode 2 to mode 1 conformation.
SODIUM CHANNEL β1 SUBUNIT mRNA EXPRESSION IN DRG NEURONS IN VITRO AND IN VIVO. Y. Oh* S. Sashihara+, K. McNabola, J. A. Black, S. G. Waxman++. "Department of Medicine, Division of Neurology, and Neurosurgery, University of Alabama, Birmingham, AL 35294; and "Department of Neurology, Yale University School of Medicine, New Haven, CT 06510. The voltage-sensitive Na+ channel β1 subunit (Naβ1) mRNA has recently been localized in the developing and adult rat dorsal root ganglion (DRG) neurons. This mRNA, which is expressed in the adult heart, is expressed temporally and spatially. In the present study, we have examined the expression of Naβ1 mRNA in adult rat dorsal root ganglion (DRG) neurons using in situ hybridization and in vitro radioactive in situ hybridization cytochemistry. At 3-4 days in vitro, Naβ1 mRNA was prominent in DRG neurons. The level of Naβ1 expression appeared to be increased in medium-to-large (>25 μm) DRG neurons than in small (<25 μm) DRG neurons. The Naβ1 mRNA was localized to the somata of the DRG neurons and was not detectable in the neurites. In intact DRGs, results were consistent with those in vitro studies. The level of Naβ1 mRNA expression was higher in intermediate-to-large (>25 μm) DRG neurons compared to that in small (<25 μm) DRG neurons. This cell body size-related Naβ1 mRNA expression is consistently observed beginning at postnatal day 4 and continues throughout development to adulthood. These results demonstrate that Naβ1 mRNA is expressed in PNS neurons in vitro and in vivo, and suggest that Naβ1 gene expression in DRG neurons is differentially regulated in relation to their cell body size. [Supported in part by the VA, NIMH and NIH]
SODIUM CHANNELS IV


The kdr trait of the house fly (Musca domestica), which confers reduced neuronal sensitivity to DDT and pyrethroid insecticides, exhibits tight genetic linkage to RFLP markers lying within the sodium channel gene that is homologous to the para gene of Drosophila melanogaster. The coding sequence of the house fly para gene was determined by direct automated DNA sequencing of PCR fragments obtained by amplification on first strand cDNA from adult heads. The predicted house fly amino acid sequence was 90% identical to that of D. melanogaster para gene product. In contrast to the multiplicity of sodium channel isoforms that result from alternative exons usage in D. melanogaster, there was no evidence for the existence of multiple splice variants among the cDNAs obtained from adult house fly head preparations. The house fly para sequence identified in this study was most similar to the [a*{b+c-def}]* splice variant of D. melanogaster, which occurs as a minor variant in adults but not embryos. However, the house fly sequence also contained segments located within the region of anticipated alternative splicing that do not correspond to any of the identified alternative exons from D. melanogaster. Comparison of para coding sequence for the D. melanogaster (NaiD susceptible) and kdr (resistant) house fly strains revealed several nucleotide substitutions that result in changes in amino acid sequence. The role of these sequence polymorphisms in conferring the kdr insecticide resistance phenotype remains to be determined.

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION II

171.2 DEVELOPMENT OF K+-LIKE CURRENTS IN ACOUSTIC-VESTIBULAR NEURONS OF THE CHICK EMBRYO BRAIN STEM IN VITRO K.R. Hendrix, W. Ann Housain, D.A. Sosonko, J.M. Kwan, and W.A. Amin*. Dept. of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

The show-like mammalian Kv1.1 channel is a voltage-dependent potassium channel, highly expressed in mature auditory brainstem nuclei. Associated currents have a high activation threshold, a sustained inactivation and a very short time-to-half-activation. We have used a temporal expression system to characterize these currents expressed in HEK293 cells. In addition, we have examined the effects of drugs that modify K+ channels on these currents. The results indicate that the Kv1.1 channel is a potential pharmacological target for the treatment of hearing loss.


The mouse delayed rectifier potassium channel mKv1.1 is widely expressed in the CNS and elsewhere, and is thought to be important in normal neuronal functions such as axonal conduction. In order to assess the consequences of the absence of this channel, the mKv1.1 gene open reading frame (ORF) was removed through targeted homologous recombination. A targeting construct was built containing 129/Sv genomic DNA sequences flanking the mKv1.1 gene ORF, a neomycin resistance cassette, and a thymidine kinase gene. The construct was electroporated into cultured embryonic stem cells, which were then subjected to both positive and negative selection. Those of the ES cells clones which screened positively by Southern blot for having undergone homologous recombination were injected into blastocysts for production of chimeras. Chimeric mice from two clones proved capable of germine transmission. The chimeric founders have been mated with Black Swiss, 129Sv and CS7B6 mice, and homozygotes have been obtained from heterozygote intercrosses. Use of an mKv1.1 ORF probe on a Southern blot has confirmed the absence of the mKv1.1 gene ORF in homozygous animals. The homozygotes show increased mortality and a phenotype with possible motor and/or neurological abnormalities. Studies are underway to elucidate the physiological basis of the apparent phenotype.

171.4 TWO DISTINCT, OVERLAPPING "DELAYED-RECTIFIERS" DETERMINE THE VOLTAGE-DEPENDENT POTASSIUM CURRENT PHENOTYPES IN ST. LACONLORMM MOLECULAR INTEURIONS IN PRIMARY CULTURE. A. Chua, B. Lepock, T. McCallum, NICHOLCM, NM, Bethesda, MD 20024.

Whole cell, voltage-clamp recordings were made from primary cultures of dissociated st. l. M interneurons from rats P5-P10. In cells with a bipolar morphology, "delayed rectifier" outward K+ currents were activated at test potentials positive to -40mV (Vh = -40mV). One of two current phenotypes was usually observed which we termed "slowly-inactivating" and "slowly-deactivating". The voltage-dependence of activation of either current phenotype, however, was fit by the sum of at least two Boltzmann equations suggesting that the total outward current resulted from the temporally and/or spatial overlap of components. The slowly-inactivating component showed minimal (≈20%) voltage-dependent inactivation (Vh = -65 ± 3.8 mV, n=14) in cells possessing predominantly "slowly-inactivating" outward currents, 4-AAP dose-dependently (10μM - 30μM) blocked the total outward current (Vh = -105 ± 10 mV, n=12), but not the slowly-deactivating component. At a maximal concentration of 30μM, 4-AAP selectively blocked 41% of the total current, isolation of the 4-AAP sensitive component yielded a "slowly-deactivating" current with a positive voltage dependence of activation (Vh = 11.9 ± 4.1 mV, n=6). The remaining 30μM 4-AAP was "sustained" and also possessed a positive Vh of 62 ± 2 mV, in cells where a "sustained" component was not isolated, 4-AAP (30 μM) again removed a "slowly-deactivating" component which represented only 23% (± 5) of the total outward current. TEA dose-dependently (10μM - 30μM) blocked the total outward currents with an IC50 of 142 ± 17 μM. In 30μM TEA, 95% of the total current was blocked regardless of whether the current phenotype was predominantly "sustained" or "slowly-inactivating". However, low concentrations of TEA (10 - 100μM) selectively removed the "slowly-deactivating" current component which represented 10 ± 1% of the total current. In conclusion, bipolar st. l. M interneurons have at least two "delayed rectifier" currents with similar activation profiles which can be differentiated based on their sensitivity to 4-AAP and TEA. The differing proportions of either current component usually determines the overall current phenotype in any given cell.

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**T18.5**

DO CHANNELS FORMED BY KV1.4 CONTRIBUTE TO THE TRANSIENT CURRENT IN ST. PYRAMIDAL INTERNEURONS? L. Zhang & C. McBain. NINDS-LOMN, NH, Bethesda, MD 20892.

Homomeric channels formed by the voltage-dependent KCa channel subunit KV1.4 exhibit a transient current which is inhibited by decreasing external KCa concentrations ([K+]). Immunohistochemical studies have shown that KV1.4 protein is localized to cells tentatively identified as interneurons of the CA1 hippocampus. Here we have examined the effects of [K+]o on KCa currents recorded from sulfated CA1 and compared these effects with cell morphology in slices from neonatal rats (11-15 days). With whole-cell and outside-out patch voltage clamp technique, transient currents were elicited by depolarization to potentials positive to 0 mV and were isolated from sustained currents by use of different pulse protocols. Isolated whole-cell transient KCa currents were obtained. KCa currents recorded in 3.5 mM [K+]o, 30% of all the cells recorded (n = 78) and whose morphology was subsequently identified by biocytin labeling showed a selective rundown (30%) or increase (62%) in the amplitude of the transient current measured at 100 ms. Cells were bathed with a 0.5% or 10 mM [K+]o solution, respectively. Analysis of the voltage-dependence of this transient current revealed a Vth of -20 ± 2 mV (n = 11). Of these cells, 50% were identified as inhibitory interneurons located close to the border of the pyramidal layer, 17% were observed in the superficial stratum, and 30% were CA1 pyramidal neurons. In all other cells the transient current was increased in K+ free conditions as would be expected. Current-clamp recordings from cells exhibiting a decrease in transient current in K+ free solution in voltage-clamp studies (n = 3) demonstrated an increase in their action potential duration (18%) concomitant with an augmentation of the afterhyperpolarization amplitude (52%) in K+ free solution. In addition K+ free solution also caused a membrane hyperpolarization of ~3 mV. Our data suggest that external K+ can modulate a transient K+ current primarily arising from KV1.4 subunit expression in hippocampal CA1 neurones. This current contributes to the total transient current and participates in the action potential repolarization in inhibitory neurones of st. pyramidal.

**T18.7**

CHARACTERIZATION OF THREE DISTINCT KCa CURRENTS MODULATED BY ATP IN HUMAN NEOCORTICAL NEURONS. C.-J. Jiang & G.-G. Hsiao, Department of Respiratory Medicine, Yale University, New Haven, CT 06510.

ATP-modulated KCa channels play an important role in regulating membrane excitability and neuronal responses to metabolic stress. In order to study the presence and modulation of these KCa channels in human neocortical neurones, single channel KCa currents were recorded in the excised patch configuration from dissociated human neocortical neurones. Three outward currents sensitive to ATP were characterized. All channels were sensitive to ATP and/or PKC agonists. One of them had an unitary conductance of ~45 pS and showed a strong inward rectification with symmetric KCa across the membrane. This KCa current was inhibited by ATP with IC50 of ~150 μM and channel activity was suppressed by 4-AP and AMP-PNP and glibenclamide. The second KCa current had a conductance of ~170 pS, but no rectification was seen. This current was also inhibited by ATP, AMP-PNP and glibenclamide. Unlike the 45 pS current, however, activation of this current required the presence of μM Ca2+ on the cytosolic side. The third KCa current was Ca2+ dependent, had a conductance of ~260 pS and was inhibited by charybdotoxin. Unlike the other 2 channels, ATP (0.03-1.0 mM) enhanced channel open probability and increased the unitary conductance. These results indicate that a number of KCa channels are regulated by physiologic concentrations of ATP in human neocortical neurones. (Supported by NIH P20 NS25758, HD15736)

**T18.8**


Intracellular recording techniques were used to investigate the interaction between the metabotropic glutaminergic and α2-adrenergic signaling pathways in pyramidal cells from the CA1 region of adult rat hippocampal slices. Both trans-1-aminocyclopentane-15,35-dicarboxylic acid (ACPD) and 2-chloro-6{-[2-[4-3H]aminoethyl]benzene-sulfonamide (ISO) blocked the slow afterhyperpolarization (aHP) which followed membrane depolarization when applied individually. However, their effects were less than additive when applied in combination. The effect of the combined agonists became additive when potassium channel 1 (PC1) was inhibited by PC19.31, in the recording electrode. The involvement of PC11-conformational effect of chloride, a specific inhibitor of PC1, which also rendered the combined effect of ISO/ACPD additive. Interestingly, the inhibitory effect of ACPD on aHP was not blocked by charybdotoxin, which did prevent the blockade of the AHP by photo-dissociated charybdotoxin (PDBu). The ISO-ACPD interaction could be mimicked by the combination of ISO and PDBu, and most importantly, by 8-bromo-CAMP and PDBu. These results indicate that the metabotropic glutaminergic pathway coupled to PC1 renders the α2-adrenergic inhibition of the aHP.

The demonstration that PC1 can prevent an effect of the PKA pathway reveals a potentially important mode of interaction between these two pathways, expressed as an assignment of rank to different neurotransmitters. Thus, a neurotransmitter of a higher rank (glutamate, coupled to PC1) can override the effect of a lower ranking transmitter (norepinephrine, coupled to CAMP), enabling the neuron to discriminate among neurotransmitters released in temporal proximity. Such interacting effects on the AHP might function as a protective mechanism, preventing over-simulation of pyramidal neurones upon release of multiple excitatory neurotransmitters. Supported by NARSAD and a VA Merit Grant.

**T18.9**

Properties of Ca2+-dependent, large conductance KCa channels in cultured rat melanotropes. S.J. Kahre*, K. Khelani, M. Milton, and K. Wong, Department of Physiology, UBC, Vancouver, B.C., Canada, V6T 123.

The biophysical and pharmacological properties of KCa channels were studied using the patch-clamp technique. In excised patches the open probability (Popen) was increased by depolarization and/or by increasing [Ca2+]. The current/voltage relationship in symmetrical 150 mM KCa was linear between -50 and 60 mV and had a slope of 267 pS (95% C.L. 258-275 pS). Relative permeabilities, estimated from the reversal potential obtained under either directly or by extrapolation in biionic conditions in which the test current completely replaced internal KCa, gave a permeability sequence of KCa > Rb > Na > external KCa > Na > Ca ( <0.2). Under Rb+ currents, a membrane capacitance of 30-40 pF suggesting a long mean dwell time for Rb+ in the pore. Outward currents were not seen with either Ca2+ or Na+. External TEA+ caused a weakly voltage-dependent open channel block. The K1, for the block at 0 mV was 260 μM and the slope of the Hill plot was 0.82 implying that the block was produced by the binding of a single molecule of TEA+. Internal TEA+ (80 μM) had no effect. External charybdotoxin (10-40 μM) caused a slow block of KCa channel, stocchiometrically Popen with outside-out patches precluded a systematic study of the binding kinetics of charybdotoxin.

**T18.10**


Patch-clamp techniques were used to study the calcium-activated potassium currents (RAKC) of retinal ganglion cells (RGCs) recorded by prior injection of thorium I, latex beads. In the cell-attached configuration, with 140mM K+ in the electrode and 5.9mM K+ in the bathing solution, two types of calcium-activated potassium channel were identified. These channels had conductances of 118 pS (BK) and 23 pS (SK) and were sensitive to intracellular calcium concentration resulting from the addition of Bay-K8644 to the bathing solution. In the inside-out configuration the open probability of both channels increased as intracellular patch calcium concentration was increased. In the patch-clamp mode, bath application of charybdotoxin, a selective blocker of BK channels, significantly increased the firing frequency of RGCs which responded in sustained manner to maintained depolarization. In contrast, charybdotoxin had little or no effect on the discharge patterns of RGCs which responded to such current injections in a transient fashion. These results demonstrate that the BK channel of the calcium-activated potassium conductance regulates the firing frequency of sustained RGCs. Supported by NIH grant EY-03991 to LMC.

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WEDNESDAY PM

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION II

1825
118.11


ATP-sensitive K+ channels (KATP) are the most abundant K+ channels in skeletal muscle, and may differ significantly in their metabolic actions. An abnormal functionality of KATP channels has been proposed to contribute to the pathogenesis of hypokalemic periodic paralysis (HPP). The same primary cause may involve a mutation of one of the 2-KATP receptor (Kir6.2) subunits. KATP receptors are composed of two different Kir6.2 subunits and two different sulfonylurea receptor (SUR) subunits. At present, the link between the channel mutations and the etiologic change in the ion channels is not known. Two different HPP receptors have been identified in the same patient, and both represent a new class of KATP receptor. The work was carried out in cooperation with a research group at the University of Alabama at Birmingham, Alabama, USA.

118.12

DEPRESSION OF POTASSIUM CURRENT BY 5-HT7 RECEPTOR STIMULATION IN CULTURED RAT SUPRAECHASMATIC NUCLEUS (SCN) NEURONS. Y. Fujita, T. Fujita, K. Takehara, H. Ishida, M. Hoshi, and N. Ishii. Dept. of Pharmacology, Fac. of Pharmaceutical Sci., The Univ. of Tokyo, Bunkyo-ku, Tokyo113, Japan.

The supramacular nucleus (SCN) receives a serotonergic projection from the mesencephalic raphe nuclei and this input is thought to be important in circadian rhythms regulation. We performed the whole-cell recordings from the cultured postnatal rat SCN neurons to investigate the possible modulation of delayed rectifier potassium current (IK) by serotonin (5-HT). Sustained outward current induced by depolarization to 0 mV by pulse stimulus for 500ms was identified as IK, since it was greatly inhibited by tetrodotoxin treatment. Administration of 5-HT depressed IK in a dose-dependent manner (10-6 to 10-3 M, B-OH-DPAT (5-HT7), 10-3 to 10-4 M and 5-HT(1A/1B, 7 agonist, 10-2 to 10-4 M) had similar effects of depression of 5-HT, but DDI (5-HT2 agonist, 10-3 M) wais no significant effect. Rilanserin (5-HT2 antagonist, 10-6 M) inhibited the suppressive effect by B-OH-DPAT (10-3 M), whereas pindolol (5-HT1A/1B antagonist, 10-2 M) did not. The effect of 5-HT (10-3 M) was also inhibited by rilanserin (10-4 M), but not by pindolol (10-3 M) or MDL72222 (5-HT4 antagonist, 10-4 M). Forskolin, adenylate cyclase stimulator, and 8-Br-cAMP, membrane-permeable cAMP analog, also reduced the IK. The effect of 5-HT and forskolin were not additive. Moreover, the inhibitory effect by 5-HT was attenuated by H-6, potent inhibitor of protein kinase A, to indicate that the depression of IK is induced by the stimulation of 5-HT7 receptors, which is linked to intracellular cAMP-coupled mechanisms.

118.13


Myelinated rat dorsal root ganglia have a variety of kinetically fast and slow potassium channels (Roper, J. and Schwartz, J.R. 1989) but little is known about the K+ channel organization of the cell bodies of these axons. Recently, several workers have identified that dorsal root ganglia neurons contain slow, voltage-dependent K+ channels (DRG) neuron changes in response to axotomy. The present study was undertaken to characterize K+ currents on the cell bodies of DRG neurons as a precurser examination of the excitatory-induced plasticity on DRG neurons. DRG neurons were dissociated from adult female Wistar rats and prepared for short-term (10-24 hrs) examination. Whole cell patch clamp recordings were obtained from medium sized neurones in the DRG. Single K+ currents were isolated by blocking Na+ and Ca2+ currents with appropriate ion replacement and channel blockers. Single K+ channel currents were 15-600 pA at 70 mV step (100 ms duration) increments to +20 mV. The beta-adrenoceptor-sensitive, nonactivating inward-outward delayed outward current was observed, and was observed by leak conductance 50% of the fast K+ current at 0 mV. Inactivation of the high voltage-activated K+ current was observed by the voltage clamp protocol or those designed specifically to identify A-current. Intra-cellular recordings from myelinated sensory axons in the root revealed a prominent 4-AP sensitive after-hyperpolarization suggesting the presence of fast voltage-sensitive K+ currents on these axons. The presence of this prominent 4-AP sensitive current on these axons and their absence or paucity on the cell body suggests that adult DRG neuron primarily display fast K+ currents on their axons and not their cell bodies. Supported in part by the VA and the NIH.

118.15


Whole-cell patch clamp experiments were performed in lactotroph cells from primary cultures of pituitaries from lactating rats. Cells were identified as lactotrophs by staining using a prolactin antibody (NIDDK, Baltimore, MD). Cells were kept in culture for up to 5 weeks. Throughout the experiment, an inward-rectifying K current (IKr) was recorded in lactotrophs using isotonic KCI as external solution. This current differed from cell to cell with respect to current density and rate of inactivation. Inactivation studies at about -80 mV revealed 3 components of IKr current that become faster and more complete with more negative potentials. This inactivation is independent of external Na+ ion. The fast-inactivating type current in lactotrophs predominantly the inward-rectifying K+ current of GH3, rat somato-mammotropic tumour cells (Bauer et al., J. Physiol. 429:169, 1990). Changing the external K+ concentration from 150 to 15 mM (K+ 100 M) was produced using KCl produced a rightward shift in the reversal potential of 55.2 mV, demonstrating a high selectivity of the recorded current for K+ over Na+. IKr is effectively blocked by 5 mM Ba++, but 0.5 mM Ba++ was only slightly effective. The cell body current by only 10-20 mV. 100% of the current was partially (50%) blocked by Ba++.

A physiological role of IKr in the control of prolactin secretion is suggested. The TRH inhibited IKr in about half of the lactotrophs tested. This inhibition might underlie membrane depolarization resulting in a TRH-induced enhanced Ca++ influx into the cells, as has been observed in a proportion of lactotrophs (Reid et al., in prep.).

118.16

CA2+-ACTIVATED POTASSIUM CHANNELS UNDERLYING AFTERHYPERPOLARIZATION IN NEUROCHEMICAL PYRAMIDAL NEURONS. Tian Kang*, John Huguenard and David A Prince, Department of Neurology and Neurosciences, Stanford University, Stanford, CA 94304

To investigate the ionic channels underlying afterhyperpolarizations (AHPs) we utilized dual patch clamp recordings with two electrodes, a whole-cell current clamp and a cell-attached patch, on single rat neocortical neurons in visual slices. The large conductance Ca2+-activated K channel (IKCa) was identified through its contribution to afterhyperpolarization and fast afterhyperpolarization in neurons because it opens after a single spike in GH3 cells and dissociated hippocampal neurons (Lang & Ritchie, Pfizier Arch 1987; Yoshida, J. Physiol. 1982). We found that the conductance of IKCa was not triggered by depolarization induced spikes in pyramidal neurons in slices. Instead, an intermediate conductance Ca++-activated K channel (IKCa) with a conductance of 64 s was seen immediately following the AP transient and with mean post-spike channel activity lasting 90-95 ms. A small conductance Ca2+-activated K channel KCa with a conductance of 29 s (SK) was activated immediately following the AP transient in IK Ca openings, and the activity of SK last 95-96 ms. The same results were also obtained by nysting evoked spikes and at different temperatures. Spike-related openings of IK and SK were modulated by conductance of 0.3 mS in contrast to 2.5 mS in SK. In contrast, the BK channels only opened as neuronal responses dextrated eg smaller spikes and a spike-independent manner. The membrane potential became hyperpolarized (<10 mV) during BK channel activity. Inside-out patches exist from slices or dissociated neocortical pyramidal neurons also contained these types of Ca2+- activated K channels. IK and SK are more sensitive to Ca++ than BK. Similar to IK, IK was also voltage-dependent and blocked by 1 mM TEA at 100 M Charybdotoxin (CTX) in the pipette solution. SK was voltage-independent and insensitive to TEA, CNX or apamin. We conclude that IK and SK respond to the transient intracellular Ca++ elevation elicited by the action potential and contribute to the two components of AHPs. BK does not contribute to the AHP during short periods of stimulation.
POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION III

718.17
TWO CLASSES OF BK CHANNELS IN RAT NEUROEPITHELIAL CELLS. J.M. Miencke, M. Atilano, S. Mackay, B. Barker. Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

In situ patch recordings from E1-14 rat telencephalon reveal two classes of large-conductance Ca-activated K (BK) channels: the first one represents only a very small percent (~1%) of occurrences and displays properties of classical BK channels, i.e., normal gating mode, sensitivity to charybdotoxin (CTX), and an absolute requirement of Ca for activation, and voltage dependence of 10-15 mV per e-fold increase in Po. In the second class, channels exhibit a buzz gating mode and, as described previously (J. Physiol. 481: 293-298), switch to normal mode upon exposure to intracellular trypsin. These channels appear to be insensitive to CTX, are capable of activation in the absence of cytoplasmic Ca, and display voltage dependences ~40 mV per e-fold increase in Po. An interesting feature is that their Po can be "tuned-in" with intracellular trypsin, longer enzyme applications yielding lower Po's.

Following treatment, these channels are stimulated by increases in cytoplasmic Ca. Because of its overwhelming presence in embryonic tissue, this channel subclass may encompass immature forms of BK channels.

718.19

We analyzed the ion channels on neuron size and shape of the magnetic evoked fields (MEFs) in the guinea pig lateral cortical Ca3 slice. Evoked potentials (EPs) were recorded simultaneously with the MEFs to compare their similarities in timing and waveform. Intracellular recordings were carried out to gain further insight into the cellular currents generating the MEFs. The stratum lucidum of rectangular CA3 slice was stimulated by single pulses (1.0 ms, 50 μA, 0.1 - 0.5 Hz) with six pairs of bipolar electrodes in Ringer solution (concentrations in mM: NaCl 124, KCl 5, NaHCO3 26, NaH2PO4 1.2, MgCl2 0.6, CaCl2 2.5, glucose 10, 36-37°C) containing 0.1 mM phenytoin. The MEF consisted of an initial component with a peak latency followed by a biphasic slower component lasting 100 ms. 0.1 mM 4-aminopyridine (4-AP), which selectively blockes BKCa, reversibly enhanced the slower component, whereas a mixture of BKCa and EP-sensitive Ca current (TEA), which blocks BKCa, reduced the initial MEF component, but increased the amplitude of the slower component of both the MEF and EP. Blocking of BKCa (4-AP) with 1 μM cadmium was demonstrated in our preparation with intracellular recording. The same concentration prolonged the latency of the slower BKCa component with some increase in magnitude. The latency of EP response was also prolonged and its amplitude was clearly enhanced. These results indicate that: (1) the blockade of BKCa increases the duration of depolarization after the opening of BKCa thereby increasing the probability of opening KCa and consequently increasing the MEF amplitude; (2) the block of the long-lasting afterhyperpolarization may prolong the voltage-sensitive Ca2+ channel open time, thereby broadening the slower component of MEFs. We conclude that slow conductances are important in interpreting MEFs. Supported by NINDS-R01-NS21149.

718.20
HODGKIN-HUXLEY REDUX: EXCITABILITY OF THE SQUID GIANT AXON REVISITED. J.R. Clary, NINDS, NIH, Bethesda, MD 20892 and MBL, Woods Hole, MA 02543.

The Hodgkin and Huxley model (J. Physiol. 117, 500, 1952) is generally believed to provide a good description of the excitability properties of the squid giant axon. This view is incorrect. Specifically, the model predicts a steady train of action potentials (APs) in response to a sustained current pulse of sufficient amplitude, whereas the axon itself generates only a single AP for these conditions regardless of pulse amplitude or duration. The underlying mechanism for this result concerns, primarily, LK1, which requires significant revision in the model. Specifically, the LK1 current-voltage relation is a non-linear function of (V-EK), which is well described by the GKH equation (J. Physiol. 49, 444-449, 1961). This result, in turn, gives a much steeper activation curve for LK1 than in the HH model, in particular a steep rising phase at ~45 mV. Consequently, LK1 acts, effectively, as an impulse shunt during a sustained current pulse as the membrane potential is depolarized from the foot of the initial spike. LK1 is thereby shortcircuited, and the membrane quiescently rests in the -55 to -45 mV range, depending on pulse amplitude. The squid giant axon is, therefore, a good model system for rapid accommodation, rather than bursting, or tonic firing behavior.

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION III

719.1
ARE K+ CHANNEL β-SUBUNITs NADPH-DEPENDENT OXIDOREDUCTASE PROTEINS? T. McCormack, K. McCormack. 1st Dept. Physiology, Indiana University, Bloomington, IN 47405.

We have observed that Shaker K channel β-subunits (β1, β2 and β3) show homology to members of an NADPH-dependent oxidoreductase superfamily. We have also identified EST sequences from rice and Arabidopsis in which β-subunits show ~40-55% identity over the 96 amino acids encoded by these partial cDNAs. We are interested in determining whether K channel β-subunits are functional, and whether pyridine nucleotide cofactors play a role in modulating K channel channels through β-subunits. In order to pursue these studies we have cloned human homologs of the rat β-subunits. The β1 gene is complex and generated alternatively spliced products with different amino termini and tissue-specific expression. The β1 product is expressed mainly in brain, while β3 is most abundant in heart. Additional studies will focus on the physiological roles of β-subunits and on the interactions of specific α- and β-subunits.

Supported by a Grant in Aid from the American Heart Association and NIH grant NS 50989.

719.2
THE MODULATION OF HUMAN (HLA) CA2+-ACTIVATED K+ CHANNELS BY PROTEIN KINASES AND ATP ANTAGONISTS. T.J. DiChiara, P.H. Reinhart. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710 USA.

We have investigated the modulation of human K+ channels (the hH2) splice variant of hH3) in a stably transfected HEK293 (human embryonic kidney) cell line. Using inside-out patch clamp analysis we have shown that these channels can be up-modulated to a similar extent by either protein kinase A (PKA) or protein kinase C (PKC). Both of these kinases increase the steady state conductance of these channels, and also have profound effects on the channel activation kinetics (n=4). When added in the absence of protein kinases, ATP does not up-modulate hH2 channels, however, it slows channel "run-down" measured as a right-shift in macroscopic current curves (28 of 31 experiments). As soon as ATP is washed out, "run-down" continues. ATP5S has a similar, although less robust, effect which is resistant to wash-out (n=12). Three other non-hydrolyzable ATP analogs, AMP-PNP (n=11), AMP-PCP (n=3), and AMP-CPP (n=3) have no effect on the rate of run-down nor on peak currents. These data indicate that human K+ channels can be targeted by numerous signal transduction cascades. Furthermore, the data show that ATP itself also modulates hH2 channels, and thus this effect is distinct from modulation by PKA and PKC. The mechanism of ATP modulation appears to involve protein phosphorylation rather than a direct effect of nucleotide binding. Supported by NIH grant NS31253 to FHR.
719.3 REGULATION OF K\textsuperscript{+} 1.6 AND K\textsuperscript{+} 3.1 EXPRESSED IN XENOPUS OCYCTES BY PROTEIN KINASE C. A.C. Costac, L.C. Larka and P.J. Pfaffinger, Baylor College of Medicine, Houston, TX 77030.

The regulatory actions of PKC on cloned K\textsuperscript{+} 1.6 and K\textsuperscript{+} 3.1 were studied here with both electrophysiological and biochemical approaches. By using conventional whole-cell and voltage-clamp, we observed a 40-50\% reduction in peak amplitude of currents recorded from oocytes expressing either rat K\textsuperscript{+} channel clones after 15-20 minutes incubation in external solution containing phospholipid-125I substrates. In contrast, K\textsuperscript{+} 1.6 was not suppressed under these conditions. However, to avoid possible artifacts due to PKC stimulated membrane internalization, we switching to study regulation in intact external macrophages. Patches were excised from oocytes expressing either K\textsuperscript{+} 1.6 or K\textsuperscript{+} 3.1 and tested with internal solutions containing a partially purified preparation of PKC in an optimized low Ca\textsuperscript{2+} buffer. The kinase activity in this solution was 20\% of the Ca\textsuperscript{2+} stimulatable activity. Where patches could be held long enough, the current could be reversed upon kinase removal. The reversible nature of the regulation, and recording in excised, low Ca\textsuperscript{2+} solutions, demonstrates that the effect is not due to internalization. To begin to characterize the PKC sites on the channels, we have performed in vitro phosphorylation assays of a purified N-terminus fragment of K\textsuperscript{+} 1.6. This protein only shows a slight ability to be phosphorylated, around 1\% phosphorylated. We are further refining this biochemical strategy, along with mutagenesis, to better characterize the mechanism of regulation by PKC.

Supported by NIH ROI N 31583, Baylor Mental Retardation Center NIH HD-04064, Klingenstein Fellowship Award.

719.5 PHOSPHORYLATION OF CB-1 BY PROTEIN KINASE C. K. Mackie*, D.E. Garcia, A. Paradzich, P. Hilt, and B.J. Murphy, Department of Pharmacology, Physiology and Pharmaceutical Sciences, Univ. of Wash., Seattle, WA 98195-6540. [Panlabs, Bothell, WA 98011-8805.

Neurotransmitters that stimulate protein kinase C (PKC) in neurons disrupt activation of inwardly-rectifying potassium currents (K\textsubscript{r} current). In A97-20 cells transfected with rat brain cannabinoid receptor (CB-1), stimulation of PKC by pretreatment with 100 nM PMA blunted the activation of K\textsubscript{r} current: In cells treated with the inactive phosphorbid ester, 100 nM 4a-phorbol, the cannabinomimetic, 100 nM WIN 55,212-2, increased K\textsubscript{r} current (52.4 \pm 0.9 pA/pF, n=21) while in cells treated with the active analog, 100 nM PMA, WIN 55,212-2 stimulation was blunted (2.2(0.5 pA/pF, n=10). This may be a consequence of CB-1 phosphorylation. To address this issue we studied phosphorylation of CB-1-glutathione-S-transferase (GST) fusion proteins corresponding to the intracellular domains of CB-1. GST was not a substrate for PKC. The fusion protein corresponding to the third intracellular loop was heavily labeled by PKC. The first and second loops were phosphorylated to a lesser extent and the carboxy terminus was poorly phosphorylated. Phosphoserine was the only phosphorylatable residue detected. Hence, PKC stimulation blunts cannabinoid activation of K\textsubscript{r} current, and CB-1 intracellular domains potentially involved in G protein coupling are PKC substrates.

(Supported by N501588, N80174, DGAPA IN203293 and DA902093.)


719.7 EXPRESSION OF THE VOLTAGE-DEPENDENT POTASSIUM CHANNEL KV\textsubscript{1.3} DECREASES CELLULAR CYTOSOLIC TYROSINE PHOSPHORYLATION. T.C. Holmer*, K.S. Berman, M.R. Bowley and I.B. Levitan, Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Tyrosine phosphorylation modulates the properties of the Kv1.3 K\textsuperscript{+} channel. To determine whether Kv1.3 activity in turn alters tyrosine phosphorylation in embryonic kidney (HEK) 293 cells, we transfected by lipofection with cDNAs coding for control-vector, Kv1.3 and/or the constitutively active v-src tyrosine kinase. The expression of these proteins was verified in cell lysates by western blot analysis using specific antibodies. Protein tyrosine phosphorylation (PTP) in cell lysates was detected by western blot analysis using specific monoclonal antibodies. Treatment with the membrane permeant tyrosine phosphatase inhibitor pervanadate (250 \muM, 5-60 min) results in robust increases in PFP in control-vector transfected cells. Kv1.3 transfected cells showed a 40-50\% lower PFP following pervanadate treatment as compared to control cells, without any change in the protein levels of cellular kinases. Thus, Kv1.3 expression inhibits the activity of endogenous tyrosine kinases. Similar Kv1.3 induced decreases in PFP are observed in cells co-transfected to express the channel together with v-src. Transfection with a non-conducting mutant (W396F) Kv1.3 channel does not cause a decrease in PFP. Thus potassium channel activity can modulate tyrosine kinase signal transduction pathways.

719.8 THE EGF RECEPTOR TYROSINE KINASE INHIBITS THE VOLTAGE-DEPENDENT POTASSIUM CHANNEL KV\textsubscript{1.3}. M.R. Bowley*, T.C. Holmer and I.B. Levitan, Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The epidermal growth factor receptor (EGFr) tyrosine kinase is known to directly tyrosine phosphorylate a variety of targets within cells, as well as couple to several downstream serine/threonine kinase signaling pathways. We have been examining the modulation of voltage-dependent potassium channel, Kv1.3 by the EGFr cDNA's for human and rat. Kv1.3 were expressed in HEK 293 cells using lipofectamine reagent. Expression and tyrosine phosphorylation levels of Kv1.3 and other EGFr substrates were detected using biochemical techniques, while Kv1.3 activity was measured by patch-clamp recording. Co-expression of the EGFr and Kv1.3 results in a large increase in the tyrosine phosphorylation of Kv1.3, without affecting its expression. Physiologically, co-expression of the EGFr and Kv1.3 produces a decrease in peak current levels compared to control conditions. Treatment of EGFr and Kv1.3 co-transfected cells with an inhibitory EGFr ligand binding domain antibody, blocks the EGFr-induced downregulation of Kv1.3. In addition, acute EGF stimulation of transfected cells produces a decrease in peak current and some speeding of inactivation. The physiological process may be due to direct tyrosine phosphorylation of the channel, or may occur via downstream signaling events.

719.4 PHOSPHATASE 2B (CALCINEURIN) PROMOTES LOW OPEN PROBABILITY BEHAVIOR IN LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CULTURED HIPPOCAMPAL NEURONS. G.A. Hicks, B. Perrett, and N.V. Marion*. Volum Institute, O.H.S.U., Portland, Oregon 97201.

Phosphorylation and dephosphorylation can modulate the activity of ion channels. An example of this is provided by the smooth muscle channel Cav 1.2 which is dephosphorylated by calcium/calmodulin-dependent protein phosphatase 2B (calcineurin) when Ca\textsuperscript{2+} is withdrawn from the bathing solution. This effect is enhanced by ionomycin or forskolin which stimulate activation of calcineurin. In order to extend this approach to other channels, we have been examining the interaction of the activity of the large conductance Ca\textsuperscript{2+} activated channel (BK) in smooth muscle and sensory neurons. In the absence of Ca\textsuperscript{2+}, BK conductance is low, but if Ca\textsuperscript{2+} is added to the bath BK channels are stimulated, opening to a maximal conductance. The BK channel is activated by PKC. Dephosphorylation of BK channels and their activity is due to dephosphorylation by calcineurin. The level of activation of calcineurin is modulated by Ca\textsuperscript{2+} and a decrease in the Ca\textsuperscript{2+} level inactivates the channel. In contrast, Ca\textsuperscript{2+} activates the channel. Hence, removal of Ca\textsuperscript{2+} would be expected to inactivate the channel. In the present study, we examined the effect of Ca\textsuperscript{2+} removal on BK channels in the absence and presence of calcineurin. The presence of Ca\textsuperscript{2+} was examined using two methods. First, using ionomycin, a potent activator of calcineurin, BK current was reduced in a dose-dependent manner. Second, using calcineurin, BK current was decreased in a dose-dependent manner. Thus, Ca\textsuperscript{2+} and calcineurin are both required to activate the BK channel.

Supported by NIH ROI N 31583, Baylor Mental Retardation Center NIH HD-04064, Klingenstein Fellowship Award.
The DROSOPHILA SLOWpoke (dslo) CALCIUM-DEPENDENT POTASSIUM CHANNEL IS A SUBSTRATE FOR PROTEIN KINASES. J Wang* and T B Levitan, Dept. of Biochem. and Center for Complex Systems, Brandeis University, Waltham, MA 02154

The activity of the cloned calcium-dependent potassium channel dslo can be modulated by protein phosphorylation (Eggsruera et al., 1994). To determine whether dslo is a substrate for protein kinases, partial dslo sequences were expressed in E. coli as glutathione-S-transferase fusion proteins. Purified fusion protein was examined for their ability to be phosphorylated in vitro by protein kinases. One fusion protein containing dslo residues 281-933 is readily phosphorylated by both protein kinase A catalytic subunit (PKA) and protein kinase C (PKC). In contrast, a corresponding dslo fusion protein in which the residue at the position 942 (S942), within a consensus PKA site, is replaced by alanine (S942A), cannot be phosphorylated by either kinase under the same assay conditions. These results indicate that S942 can be phosphorylated by both kinases in vitro. Another fusion protein containing the last 828 residues (337-1164) of dslo, a proposed cytoplasmic domain of the channel protein, can also be phosphorylated by both kinases. An S942A mutation abolishes the phosphorylation by PKA, suggesting that S942 is the only target for PKA in this long stretch of protein sequence. However, the same mutant protein can still be phosphorylated by fusion proteins indicating the presence of other serine or threonine residues accessible to PKC.

11.11 EFFECT OF DOCOSAHEXAENOIC ACID ON mKv1.1 AND mKv1.2 K+ CHANNELS. Jennifer C. Garratt, Matthew P. M. Evoy and David G. Owen* Molecular Pharmacology, Wyeth Research, Taphlow, Berks, U.K.

Docosahexaenoic acid (DOHA) is a long chain polyunsaturated fatty acid which is abundant in fish oil. External administration of DOHA caused a time-dependent block of the cardiac potassium channel, Kv1.2 (Henneker et al., 1994). Using whole-cell patch clamp, the potassium channel-blocking effects of DOHA were examined on two cloned mouse brain potassium channels, mKv1.1 and mKv1.2 stably expressed in Chinese hamster ovary cells. Transfected cells expressed a non-inactivating, "delayed rectifier"-type potassium channel; mKv1.2 has a slower activation rate. For purposes of quantifying dose-dependent block of these currents overall charge transferred during the voltage step (Qton) was measured. External administration of DOHA produced a dose-dependent, reversible block of mKv1.1, (EC50 = 7.5uM) and mKv1.2 (EC50 = 2.1uM). Blockade of these channels is time-dependent eg at +70 mV for mKv1.1 DOHA (15uM) the time constant of deactivation (tau) was 52.56±4.9msec and for mKv1.2 DOHA (15uM) it was 11.6±0.34msec. The time course of the DOHA induced decay of mKv1.1 and mKv1.2 decreased in a concentration-dependent fashion. Interestingly DOHA was found to increase the rate of activation of mKv1.2 (0.857±0.02ms) compared with control value 1.78±0.14ms. Internal administration of DOHA (300µM) did not alter either mKv1.1 or mKv1.2 current. These findings suggest that DOHA blocks both mKv1.1 and mKv1.2 channels at a site which is only accessible via the external mouth of the channel and only once the channel is open.


A 28-mer peptide based on part of the N-terminal of the human Kv4.3 K+ channel (NHISVCGHTRFRRGERASFGCGLCIVKXK) was synthesized (X = K channel, Kv1.1, from a non-inactivating to a rapidly inactivating current. Using the whole cell patch clamp technique to examine the "ball and receptor" model system in Chinese hamster ovary cells, we have probed some of the residues important for N-type inactivation by introducing various mutations and deletions to the hKv4.3 peptide. The sensitivity of Kv1.4 to block by modulation of the channel is critically dependent on the cysteine at position 6 (Stephens & Robertson; J. Physiol. 484, 1-13). Cysteine to serine substitutions destabilised the inactivated state of the mouse Kv1.1 peptide channel, but did not abolish inactivation. Control time constant of recovery from inactivation (tauir) at -90 mV was 1.5 ± 0.1 s; (n=9); this was faster than the mutants C65 (0.2 ± 0.4 s; n=5), C65 (0.6 ± 0.1 s; n=5) and C65, C65 (0.9 ± 0.2 s; n=7). The apparent K0 (control = 8.5 ± 1.2 uM) was most drastically reduced by the double mutant C65, C65 (4.9 ± 0.5 uM; n=5) and C65 and C24 (11 ± 2 uM; n=5), but not by C65 (12.5 ± 2 uM; n=5). Deletion of the five residues preceding C6 (Δ1-5) reduced the apparent affinity of the peptide (K0 = 33 ± 12 uM; n=7); this deletion most noticeably affected association (K0) with the receptor (Δ1-5; n=7) being reduced to 0.05 ± 0.01 uM/°P ( n=4) for Δ1-5. In contrast, deletion of the four residues after C24 (Δ25-28) caused an increase in K0 to 0.78 ± 0.16 uM/°P (n=8); Δ25-28 had a K0 of 6.2 ± 4 uM (n=8). The results indicate that neither cysteine residue is essential for N-type inactivation, but that their presence does stabilise the inactivated state and that residues closest to the N-terminal may be important for occupation of the ball receptor. It is hoped that such studies will reveal more about the processes of inactivation, and possibly its potential for modulation.

11.14 REGULATION OF POTASSIUM CHANNEL EXPRESSION IN DEVELOPING GNNRH NEURONS BY FIBRONECTIN CONTACT. Martha M. Bona* Dept. Pharmacology, Univ. Washington, Seattle WA 98195. GnRH neurons in the adult animal are situated in the hypo-thalamus, but are embryonically derived from the peripheral olfactory placode, making it in which to study CNS neurons during migration and development. GnRH neurons were enzymatically dissociated from olfactory placodes isolated from 11.5 mouse embryos, and cultured using whole cell patch clamp, and cells identified by immunocytochemistry. Isolated GnRH cells were compared with those in contact with Fibronectin, increased in size (measured by cell capacitance) with time in culture from 2.8±0.2 pF (n=18) on day 1 to 11.8±2.7 pF (n=4) on day 5. From days 1-5 peak outward current increased, but remained low due to the increase in cell size (day 1, 10.6±1.8 pA/PF; day 5, 6.3±1.8 pA/PF). 50% of these isolated cells expressed a small transient inward current. In contact cells, outward currents were larger on day 2 (15.8±2.7 pA/PF, capacitance 6.6±1.3 pF, n=5), and increased on days 3-5 (day 5, 35.5±9.1 pA/PF, capacitance 6.5±0.6, n=4). In addition, these cells expressed a large transient inward current. Immunocytochemistry with an antibody specific to the Shaker subfamily potassium channel mKv1.1 demonstrated expression of this protein in the isolated GnRH neurons. Supported by the Andrew W. Mellon Foundation.

11.15 ACCESS OF SUBSTANCES TO POTASSIUM CHANNELS IN XENOPUS OCYTES: INFLUENCE OF FOLLICULAR TISSUES. M. Mageda*, U. Mullbott and E. H. Steckmann, Institut für Physiologie, Robert-Koch-Str. 27a, D-81419 München, Germany.

Oocytes of Xenopus laevis are a widespread model system to express and characterise neuronal ion channels and receptors. Concerning the investigations of these model systems, however, it is uncertain, if follicular tissues reduce the access of substances to the molecules expressed. In order to shed some light on this, the effects of various blocking agents on voltage-operated and voltage-independent channels were studied in oocytes with and without follicular tissues. The experiments revealed the following: (i) Each tested blocking agent was more effective when applied to follicular tissues. This was found true for ions (lead, barium), smaller molecules (TEA, 4-AP, diltiazem, nifedipine, verapamil, pentleytenestrol) and large molecules (DXT, MCDP; n = 9 to 21 each). (ii) The concentration-response curves were shifted for follicular tissues. The IC50 values in oocytes with and without tissues were 4.1 and 1.0 mM for TEA and 30.0 and 1.2 mM for lead, respectively. (iii) The times needed for the half-maximal blocking effect to reduce in oocytes without follicular tissues. The IC50 values in oocytes with and without tissues were 0.33 and 0.07 s for TEA, 8.8 and 2.4 s for DXT and 4.2 and 0.2 s for lead, respectively. On a whole, the follicular tissues appear to reduce the sensitivity of the potassium channels to blocking agents. This 'barrier effect' has to be considered in pharmacological work on oocytes.
720.1
Wyeth-Ayerst Research, CNS Disorders, Pearl River, NY 10965.

Alcohol-dependent potassium (K+) currents associated with Kv1.1, Kv1.2 and Kv1.6 voltage-gated K+ channels. The rank order of potency determined by electrophysiological technique in clonal cell lines expressing Kv1.1 was: Kv1.2 > Kv1.1 > Kv1.6. Furthermore, biochemical studies have revealed that Kv1.2 is the major component of the DTX-sensitive receptor in brain. The present study characterized the radioligand binding profile of [125I]-DTX to the rat Kv1.2 subunit stably expressed as a homomeric channel. The [125I]-DTX showed 50% inhibition at 1.2 ± 0.05 ìM, a significantly lower IC50 value than Kv1.1 (9.7 ± 0.2 ìM) and Kv1.6 (33 ± 3 ìM). The rank order of potencies for Kv1.2 channel blockers was: Margatoxin (0.016 ± 0.012 ìM) > Stichodactyla Toxin (30 ± 11 ìM) > DTX (24 ± 8 ìM) > Charybdotoxin (CTX; 23 ± 3 ìM) > Mast Cell Degranulating Peptide (986 ± 66 ìM). Interestingly, the Hill coefficient for DTX was significantly closer to unity in the Kv1.2 cell line as compared to previous results in rat brain. Although Kv1.2 plays an important role in the DTX-sensitive subunit in brain, the pharmacological profile of [125I]-DTX binding differs significantly between brain and the clonal Kv1.2 cell line.

720.2
BEHAVIORAL EFFECTS OF THE POTASSIUM CHANNEL BLOCKER 4-
Wyeth-Ayerst Research, CNS Biology, Pearl River, NY 10965.

Biochemical and electrophysiological studies have demonstrated that 4-aminoypyridine (4-AP) and its structural analogs are blockers of voltage-dependent potassium (K+) channels. However, few studies have evaluated the behavioral effects of this group of compounds and several studies have suggested that many of the analgesic effects of 4-AP are not mediated by K+ channel blockers. Therefore, research was conducted to determine whether 4-AP and its analogs have similar behavior effects in rodents that are likely to affect their actions at CNS K+ channels.

720.3
ANTIDEPRESSANT-LIKE EFFECTS OF POTASSIUM CHANNEL BLOCKERS.
Wyeth-Ayerst Research, CNS Biology, Pearl River, NY 10965.

The antidepressant-like effects of the potassium channel blockers 4-aminoypyridine (4-AP) and quinidine were compared with those of two animal models (tail suspension test, forced swim test, and DRL-72 sec schedule) that are sensitive to clinically effective antidepressants. For the tail suspension test, mice were suspended, with their tails and the bottom of the swimming area scored during a 6 min test session. The forced swim test, rats were placed in a cylinder of water for a 15 min swim test. A subchronic dosing procedure was used, with injections occurring at 30 min intervals. The following were used to test the effects of the drugs. At the 24th hour, rats were placed in a cylinder of water and the duration of immobility was scored during a 5 min swim test session. For the DRL-72 sec schedule, rats were trained to lever press for a food pellet. After lever pressing, a response was reinforced only if it occurred 72 sec after the previous response or the start of the session. Both 4-AP and imipramine produced antidepressant-like effects in all three animal models. A subchronic dosing procedure decreased immobility time in the forced swim and tail suspension tests. In addition, both 4-AP and imipramine dose-dependently increased the number of rejections caused and produced modest decreases in response rate in rats responding under a DRL-72 sec schedule. Quinidine produced antidepressant-like effects in two of the three animal models. Quinidine produced dose-dependent decreases in immobility time in the forced swim test, but did not affect immobility time in the tail suspension test. Quinidine also produced dose-dependent increases in the number of rejections caused and produced modest decreases in response rate in rats responding under a DRL-72 sec schedule. The present results suggest that K+ channel blockers may have therapeutic potential as antidepressant drugs.

720.4
Wyeth-Ayerst Research, CNS Disorders, Pearl River, NY 10965.

Previous studies have shown that the potassium channel blocker 4-aminoypyridine (4-AP) induces wet dog shakes, head thrust and head movements, and tremors in rats. Since these behaviors resemble those produced by 5-HT1A agonists, these studies were conducted to determine whether 4-AP-induced behavioral changes were mediated by 5-HT1A receptors. In the first study, rats were pretreated with the 5-HT1A receptor antagonist ketanserin (3 mg/kg) 30 min prior to administration of 4-AP (1 or 3 mg/kg) or saline (S). During the 30 min post-injection period, 4-AP increased the number of wet dog shakes (Mean±SEM: 3S=0, 1 mg/kg=14±6, 3 mg/kg=14±5) and the number of rapid repetitive forelimb movements (S=0, 1 mg/kg=19±8, 3 mg/kg=14±8). Ketanserin pretreatment attenuated both 4-AP-induced effects (Wet dog shakes: S=3, 1 mg/kg=4±3, 3 mg/kg=6±2; Repetitive forelimb movements: S=3, 1 mg/kg=22±1, 3 mg/kg=6±4). In the second study, 5-HT1A receptor binding was determined. 5-HT1A receptor binding was not affected by 4-AP pretreatment. In a third study, the effect of 5-HT1A receptor antagonists on the 4-AP-induced behavioral changes was determined. 5-HT1A receptor binding was not affected by 4-AP pretreatment. In conclusion, these results indicate that 4-AP-induced effects may be mediated by 5-HT1A receptors. These results are consistent with the in vivo results of Schecter (this meeting) who found that 4-AP stimulates 5-HT[3]-HT release. Taken together, these results suggest that K+ channel blockers modulate serotonergic neurotransmission.

Using cell-attached patch-clamp recordings from freshly dissociated rat corpus striatum neurons, we are studying the blockade properties of an inwardly-rectifying 80 pS K⁺ channel, which is activated by DA-like dopamine receptors. Several previous studies showed that this channel was sensitive to sulfonamides drugs, and was blocked by nanomolar concentrations of quinolone and related quinolinedine amines. Here, we found that both tetraethylammonium (TEA, 5 mM) and 4-aminoypyridine (4-AP, 1 mM) partially blocked this channel when applied to the external surface of the membrane via the patch pipette. Block was relatively voltage-insensitive, and was accompanied by a reduction of open time and an increase of closed time. These cells also express other inwardly-rectifying K⁺-permeable channels with conductances ≈30 pS, which are dopamine-insensitive. In contrast to the 80 pS channel, these channels were largely insensitive to block by 10 mM TEA or 5 mM 4-AP. Our results indicate that the dopamine-modulated K⁺ channel is blocked by various organic amines with differing potencies, and that inwardly-rectifying ion channels of the striatum are heterogeneous in their sensitivities to blockade by these compounds.

(Supported by MH-48465.)


In the isolated carotid sinus prostacyclin enhances arterial baroreceptor (BR) sensitivity. We tested the hypothesis that the mechanism involves inhibition of K⁺ currents. BR neurons from rat nodose ganglia were dissociated and identified using retrograde label with fluorescent dye (Di) applied to the aortic arch two weeks earlier. Whole-cell outward K⁺ current was recorded with standard patch-clamp techniques and measured at 200 ms after depolarization from -80 to +40 mV. Drugs were delivered by exchanging the bath solution. The stable prostacyclin analog carbacyclin (100 pM) reversibly inhibited the current to 65±6% of control (n=8). C2 failed to inhibit the current in the absence of Ca²⁺ in pipette and bath solutions (n=4). Charybdotoxin (CTX; 100nM), a specific blocker of Ca²⁺-activated K⁺ current (Ica), inhibited the outward current (73±3% of control). CC also caused no further suppression after CTX (n=6). Including PKIIc, a specific inhibitor of protein kinase A, in the pipette solution abolished the inhibitory effect of CC (n=6). We conclude that prostacyclin inhibits the CTX-sensitive Ica in BR neurons through a protein kinase A-dependent pathway. This mechanism may contribute to the sensitization of BR by prostacyclin.

MECHANISMS OF DihYDROPyRyDINE Block Of SHAKER POTASSIUM CHANNELS. A. Kamath, K. Lamun, F.F. Sobata*, and T. Hoshi. Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, Iowa 52242.

Dihydropyridines (DHPs) are well known as Ca²⁺ channel agonists and antagonists. Recent evidence indicates that DHPs also affect voltage-dependent K⁺ channels. Thus, we examined how DHPs affect Shaker K⁺ channels expressed in Xenopus oocytes using the patch-clamp method.

Nicardipine, nicardipine-nisoldipine, and Bay K 8644 at 10 to 100 μM induced a time-dependent reduction of the K⁺ currents of mutant Shaker channels with disrupted N- and C- type inactivation (Shibikawa et al., 1989). The time course of the current decline induced by the DHPs was approximated by one exponential. When applied in a sequential manner, the onset of the current reduction was rapid and the effect was reversible. Single channel analysis showed that the DHPs reduced the mean time in a concentration-dependent manner without affecting unitary conductance.

Efficacy of the DHPs was dependent on residue 463 located in the 56 segment. This residue has been shown to influence C-type inactivation. The estimated DHP off rate was much slower with nocistatin at this position than with alanine. Since the DHPs induced an apparent inactivation, we investigated whether they compete with N- and C-type inactivation. The results suggest that the DHPs do not compete with either inactivation and that they may work to accelerate the intrinsic inactivation rates.

Structural groups on different DHPs crucial in reducing K⁺ channel currents were also examined. The estimated off rate was greater for nifedipine and Bay K 8644 than Yohimbine, suggesting that the side group at C3 may be important in determining the DHP efficacy. (Supported by American Heart Association Iowa Affiliate, and Klingenstein Fellowship to T.H. and an Established Investigatorship award from the AHA to E.F.S.)

NITRIC OXIDE ACTIVATES CALCIUM-DEPENDENT POTASSIUM CHANNELS IN CEREBROVASCULAR SMOOTH MUSCLE CELLS. C. Chen, D.A. Muth*, Dept. of Physiology, Faculty of Medicine, Univ. of B.C., Vancouver, B.C., Canada, V6T 1Z3

Nitric oxide is probably the major endothelium-derived relaxing factor (EDRF). It is thought to relax smooth muscle cells by stimulation of guanylate cyclase via its product cyclic GMP and GMP-dependent modification of several intracellular processes, including activation of potassium channels through GMP-dependent protein kinase. Here we present evidence that sodium nitroprusside (SNP), a vasodilator that decomposes into nitric oxide, can directly activate large conductance calcium-dependent potassium channels (Kc) in cell-free, inside-out membrane patches excised from cerebrovascular smooth muscle cells of adult rats.

The Kc channels studied here showed the conductance of 203±8 pS (mean ±s.e., n=15) between -80 and +80 mV in symmetrical 140 mM KCl solutions. Bath solution contained (in mM): 140 KCl; 2.6 CaCl₂; 10 HEPES; 3 EGTA (free calcium). Patch pipette solution contained (in mM): 140 KCl, 1.48 CaCl₂, 10 HEPES, 3 EGTA. Application of SNP (100 μM) to the bath solution enhanced the Kc channel open probability by 1.610 ±1.2 times (mean ±s.e., n=15), particularly the mean closed time of the channels was 0.65±0.05 ms (mean ±s.e., n=15) that seen in control solution. However, no difference was found between the conductances of SNP activated and control Kc channels.
POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION IV

WEDNESDAY PM

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION IV

WEDNESDAY PM

11:00 AM

Gangliosides (neuronal and non-neuronal) and their role in synaptic plasticity.


11:15 AM

The role of the potassium channel in the regulation of neuronal excitability.

S. J. Waxman and J. M. Miller.

11:30 AM

The effects of potassium on neuronal activity and synaptic transmission.


11:45 AM

The role of potassium channels in the regulation of neuronal death.


12:00 PM

The role of potassium channels in the regulation of neuronal membrane potential.


12:15 PM

The role of potassium channels in the regulation of neuronal plasticity.

ACETYLCHOLINE RECEPTOR: NICOTINIC—PHARMACOLOGY

271.1

Functional Pharmacology of Neuronal Nicotinic Acetylcholine Receptors (nAChRs) from the Human Cell Line IMR-32

Nelson, M.E., McCaughey, V., Chiu, A.L., and Lefkowitz, R.J.

Dept. of Neuroscience, Univ. of Pennsylvania Medical School, Philadelphia, PA 19104

The IMR-32 cell line is a human neural crest-derived cell type that has been shown to express neuronal-type AChRs including α3, α4, α7, β2, and β4. Using single-channel electrophysiology in Ca2+-free solutions, we have characterized the currents elicited by the two major subtypes, ACh and tetracaine (TM). All agonists with similar efficacies and their respective EC50 values were approximately 7.6, 22.5, 31.2, 290, and 1020 μM. Currents activated by ACh (100 μM) were completely antagonized by d-tubocurarine, mecamylamine, and dihydro-β-alanine (DHPA). The EC50 values for α3, α7, and 8.5 μM, respectively, while methylcyclizine (up to 1 μM) was virtually ineffective. Single-channel currents activated in outside-out patches from IMR-32 cells had a chord conductance of 46 pS and a linear range of channel open times that best fit double exponential functions with time constants of 1.7 and 60 μs. Comparisons between IMR-32 nicotinic AChR functions with those from neuronal AChRs functionally expressed in Xenopus oocytes suggest that the presence of the IMR-32 cell line is one including α3 and β4 receptor subunits, although participation of other receptor subunits cannot be excluded on the present data. (Supported by grants from NINDS, STJR, and MDA.)

271.2

Galanthamine is a positive modulator of the α-Bungarotoxin-sensitive hippocampal nicotinic receptor activity. P. G. Almeida, J. R. C. Albuquerque, and A. M. P. D. Pires.


A new binding site has been described using muscarinic nicotinic receptors (nAChRs) through which the iso-channel can be activated (J. Pharmacol. Exp. Ther. 265: 1474, 1993). This site is referred to as the phystostigmine (PHY) site, is insensitive to the α7 agonists such as ACh and nicotine (α1-AX), and recognizes agonists anti-cholinesterase PHY and galanthamine (Gal). To determine whether the nAChR activity induced by classical nicotinic agonists and this novel PHY site used the whole-cell mode of the patch clamp technique to investigate the effects of eugenol on β-galactosidase (β-gal)-sensitive current elicited by 500-μs pulses of ACh. In 2103 kcsa cultured hippocampal neurons, the ACh induced an increase in the peak amplitude by 50% over control. With a peak amplitude of about 300 μA, the decay time constant was prolonged from 3.0 ms (< 100 μM) to 7.8 ms at 1 μM. These results suggest that a ligand capable of binding to the PHY site may play an important role in regulating the desensitization of the α3-β7-sensitive, presumably an α7-binding, hippocampal nAChR (U.S. Patent 5,219,596).
721.3

ACETYLCHOLINE: NICOTINIC—PHARMACOLOGY

721.4

POTENCIES OF MUSCLE RELAXANTS PANCURONIU AND (+)-TUBOCURARINE AT MUSCLE NICOTINIC RECEPTORS (nAChR).


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Clinically, pancuronium is more effective than (+)-tubocurarine (Kreg et al., 1980, Br. J. Anaesth. 52, 783-787) as a muscle relaxant. We are using the molecular basis for their action on nAChRs.

In oocytes expressing nAChRs (rat muscle, bovine, chicken, and goldfish) with pancuronium (IC50 5.0 + 1.9 pM; n=4) was more potent at blocking currents induced by 1 nM ACh than IC50 7.4 + 0.8 nM; n=4). In the rat phrenic nerve diaphragm pancuronium and IC50 2.5 nM; n=5 and 5 nM; n=5 for (+)-tubocurarine. The range of potencies is partly determined by the expression system/tissue is being investigated with comparison with effects on mouse muscle myoblasts (which also express non-competitive). The compound was used to demonstrate in intracellular Ca2+ activity (Ca2+phospho) an indirect measure of nAChR activation. 10 nM ACh increased Ca2+ from 100-200nM to 400-600nM and this was blocked by 1 nM pancuronium. We are currently quantifying the potency of ACh and pancuronium in this system.

cDNA clones were kindly donated by Professor S. Niswander, Saik Institute, California. CMG is supported by Organon Teknica, Belgium. We thank Dr R. J. Marshall and Dr A. Muir for helpful discussions.

721.5


Department of Biology, University of Utah, Salt Lake City, UT 84112.

Venomous snakes of the genus Conus produce a variety of peptide toxins which individually target a range of receptors and ion channels. Venomous snakes from the fish-hunting C. paraputeus has been fractionated and found to contain, among others, a peptide unique both structurally and mechanistically among previously characterized conotoxins. The 24 amino acid peptide was chemically synthesized and, like the native material, caused paralysis and death when injected into goldfish. Binding and photofluorophotometry labeling of the peptide to Torpedo electric organ membrane indicate that it targets the nicotinic acetylcholine receptor, but that it does not compete with a-Bungarotoxin or the conotoxin, competitive inhibitors of a-Bungarotoxin binding on the muscle subtype of the acetylcholine receptor. Electrocardiology studies suggest the peptide acts as a non-competitive inhibitor of the acetylcholine receptor by physically blocking the pore. Further analysis of analogs of this peptide will allow determination of functional groups important for the peptide-channel interaction. (Supported by NIDA grant DA-05485.)

721.7

DEVELOPMENT OF A NOVEL CLASS OF NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS: INHIBITION OF N-METHYLATED SUBSTRATE DOPAMINE RELEASE AND THE ELECTROPHYSIOLOGICAL RESPONSE OF A CLOSED NICOTINIC RECEPTOR SUBTYPE IN XENOPUS OOCYTES.


College of Pharmacy, Univ. of Kentucky, Lexington, KY 40536.

We have identified a unique non-competitive antagonist of the neuronal nicotinic receptor site. A series of N-substituted nicotine analogues were synthesized and evaluated for their ability to inhibit nicotine (10 nM)-evoked ['H]dopamine ([H]DA) release from rat striatal slices. The most potent and most effective at reducing dopamine release was 1-fluoro-3-trifluoromethylbenzyl nicotine, which was 3.5-5.5 times more potent than nicotine. The analogues were compared to the competitive nicotinic receptor antagonist desbenzyll-epibatidine (DBE). Scatchard analysis of nicotine binding revealed Ki = 1.46 ± 0.15 nM and Bmax = 117 ± 9.05 fmol/mg protein. In displacement studies, the Ki was 20.0 ± 1.5 nM for nicotine, 30.0 ± 2.1 nM for KD-DBE, and the Ki for nicotine receptor blocker, methylyamine (MDMA). The N-allyl analogues (MDMA) gave a similar pot. than MDMA and had similar efficiency to MDMA. The order of potency in the release assay was nicotine > nicotine > MDMA > MDMA > MDMA, respectively. In electrophysiological studies, MDMA blocked dopamine (DA) release from nicotine (10 nM)-evoked responses to 1 nM acetylcholine by 50% at 5 nM, and by 100% at 100 nM, and displayed no agonist activity. These data suggest that the antagonists may block the nACh2 receptor to inhibit nicotine-evoked dopamine release (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

721.8


College of Pharmacy, University of Kentucky, Lexington, KY 40536.

To study the mechanism of action of nicotine in the CNS, we have undertaken a systematic evaluation of candidate antagonists of the neuronal nicotinic receptor site. A series of N-substituted nicotine analogues were synthesized and evaluated for their ability to displace [3H]nicotine binding from rat striatal membranes. The analogues were compared to the competitive nicotinic receptor antagonist dihydro-β-erythroidine (DBE). Scatchard analysis of nicotine binding revealed Ki = 1.46 ± 0.15 nM and Bmax = 117 ± 9.05 fmol/mg protein. In displacement studies, the Ki was 20.0 ± 1.5 nM for nicotine, 30.0 ± 2.1 nM for KD-DBE, and the Ki for nicotine receptor blocker, methylyamine (MDMA). The N-allyl analogues (MDMA) gave a similar pot. than MDMA and had similar efficiency to MDMA. The order of potency in the release assay was nicotine > nicotine > MDMA > MDMA > MDMA, respectively. The possibility that the release differ from the subtypes that are primarily responsible for high-affinity [3H]nicotine binding. Thus, the analogues had marginal affinities for the [3H]nicotine binding site in rat striatum compared with DBE, implying that they do not have a significant effect on the CNS neuronal nicotinic receptor subtype, but may be selective for the 3A2 subtype (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

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**721.10**

EFFECTS OF A-85380 ON NICOTINE RECEPTOR MEDIANATED 8R\* EFFUX FROM MOUSE THALAMIC SYMPTOMATOSES. M.J.Marks and S.E.Miller.

A-85380 [3-(2S)-azetidinylmethoxy]pyridine], a novel, potent nicotinic agonist that activates a variety of human subtypes of nicotinic receptors, was evaluated for its ability to stimulate the efflux of 8R\* from sympotomates prepared from thalams of C57Bl/6 mice. The stimulation of 8R\* efflux by A-85380 was concentration dependent, but apparently biphasic. One component had a very high affinity (EC50 = 4.2 mM) and a maximal response approximately equal to that measured for 10 mM nicotine. A second component, that did not saturate by 3 \*M, was observed at concentrations above 300 nM. The 8R\* efflux measured using either 30 nM or 3 \*M A-85380 were each inhibited approximately 90% by 10 mM mecamylamine. Exposure to stimulating concentrations (0.3-10 \*M) of A-85380 desensitized 8R\* by 61%. An EC50 value about 4.5 nM was calculated for both stimulation and desensitization of efflux. Desensitization of the efflux stimulated by 10 \*M nicotine was also observed with concentrations of A-85380 that elicited an 8R\* efflux of 2.2 \*M. The desensitization rate for 0.3 \*M A-85380 (0.27 min\(^{-1}\)) was lower than the maximum rate observed for activating concentrations (2.4 min\(^{-1}\)). Symptomatoxes that had been desensitized by exposure to either nonstimulating (0.3 \*M) or stimulating (30 \*M) concentrations of A-85380 partially recovered responsiveness after the termination of stimulation (75% and 35%, respectively). The recovery from desensitization after treatment with 0.3 \*M was faster (k = 0.34 min\(^{-1}\)) than that after 30 \*M (k = 0.19 min\(^{-1}\)). The kinetics of desensitization observed after exposure to either stimulating or nonstimulating concentrations of A-85380 were comparable to those measured previously for the extremely potent nicotinic agonist, epibatidine. These studies indicate that A-85380 is among the most potent agonists yet tested at nicotinic receptors in the CNS. Supported by Abbott Laboratories.

**721.11**

CHOLINERGIC ACTIVATION OF THE ELECTROCOGTOGRAM AN AMYGDALAR ACTIVATING SYSTEM. H.Drzeniek and C.J.Vandersluis.


In urethane-anesthetized rats, 100 Hz electrical stimulation of the basal amygdala changed the slow wave activity of the neocortex (from 2-6 Hz into large irregular slow activity (LISA) to low voltage fast activity (LVFA) including frequencies of above 10 Hz). A similar activating effect was seen in the hippocampus where amygdala stimulation induced the appearance of rhythmic slow activity (RSA) in the 2-6 Hz range. This activation of neocortical and hippocampal slow wave activity by amygdala stimulation was blocked by the cholinergic nicotinic antagonist mecamylamine (150-500 \*M/kg, i.p.), but not by the peripheral antagonist methylscopolamine, in a concentration-dependent manner. In contrast, a blockade of ascending inputs from the midbrain to the neocortex by treatment with the selective serotonin receptor antagonist pirenzipine (0.5-5.0 mg/kg) or curation of the rostral midbrain did not block neocortical LISA to amygdala stimulation, even though the lesions abolished all LVFA to strong noxious stimuli such as tail pinches. Unilateral infusions of the local anesthetic lidocaine (1%) into the basal forebrain selectively blocked LISA in the neocortex ipsilateral to the infusion. However, intracerebral or systemic administration of various excitatory amino acids and antagonists (2-amino-5-phosphonopropionic acid, kynurenic acid, NPC 13626) was not effective in blocking LISA to amygdala stimulation. Extracellular single unit recordings in the basal forebrain showed that about 50% of cells that were more active during periods of neocortical LISA relative to LISA were excited by single pulse stimulation of the basal amygdala. Cells that were more active during LISA were often inhibited by amygdala stimulation. The amygdala appears to provide an important excitatory input to the basal forebrain cholinergic system involved in activation of the electrocoptogram. (Supported by NSERC, Canada).

**722.1**

SELECTIVE VULNERABILITY OF NEURONS IN THE HUMAN NUCLEUS BASALIS OF MENEY: PREDICTIVE ROLE OF AN AGED-RELATED DECREASE IN GLUR2/3 RECEPTOR SUBUNIT IMMUNOREACTIVITY. M.D.Brunovic*, R. Sheffeld, and D.M. Armstrong.

Allegheny-Pinger Research Institute, Medical College of Pennsylvania, Pittsburgh, PA 15212.

Polyclonal antibodies directed against the GluR1 and GluR2/3 subunits of the AMPA receptor complex were employed in order to examine in brains of elderly humans (average age = 75) the distribution and subunit composition of AMPA-selective receptors in mammalian neocortical neurons containing the somatostatin of Meynert (NBM). Within each case (n = 9) NBM neurons were intensely labeled for GluR1, but less so for GluR2/3. Within cases, the number of GluR1-labeled neurons was typically less than 10% of the total number of NBM neurons. This effect was not due to neural degeneration or atrophy of the regions containing the somatostatin of Meynert, as assessed by the number of neurons, the size and density of the neurons, and the number of synapses on the neurons. The decrease in GluR1-labeled neurons was also observed in all of the cases examined, and was not due to a decrease in the number of neurons, the size and density of the neurons, or the number of synapses on the neurons. The decrease in GluR1-labeled neurons was also observed in all of the cases examined, and was not due to a decrease in the number of neurons, the size and density of the neurons, or the number of synapses on the neurons. The decrease in GluR1-labeled neurons was also observed in all of the cases examined, and was not due to a decrease in the number of neurons, the size and density of the neurons, or the number of synapses on the neurons. The decrease in GluR1-labeled neurons was also observed in all of the cases examined, and was not due to a decrease in the number of neurons, the size and density of the neurons, or the number of synapses on the neurons.
AMP-SELECTIVE GLUTAMATE RECEPTOR SUBUNITS IN THE VISUAL AND CORTEX CORTICAL ORNETS OF NORMAL CONTROLS AND PATIENTS WITH TAUOPATHIES

EXCITATORY AMINO ACID RECEPTORS: RECEPTOR LOCALIZATION

272.3

AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNITS IN THE VISUAL AND CORTEX CORTICAL ORNETS OF NORMAL CONTROLS AND PATIENTS WITH TAUOPATHIES.

We used immunocytochemical techniques and antibodies against the AMPA-receptor subtypes GluR2/3 and GluR4 to examine the distribution of these receptor subunits within the visual (Areas 17 and 18) and parietal cortices of normal elderly subjects (AD). These studies are part of a continuing effort to identify selective vulnerability of glutamate receptors in AD. These regions were selected for study because they represent the most anterior (visual) and posterior (parietal) aspects of the human brain. The distribution of GluR2/3-positive receptors was examined in areas V and VI. In contrast, within area 18 both cell types were observed in areas V and VI as well as within layers II and III. These same region were examined in AD cases since significant reduction in GluR2/3 labeled neurons was observed. Within the parietal cortex, GluR2/3 pyramidal and non-pyramidal neurons were observed, with pyramidal neurons being the more abundant cell type. As in Area 18, these neurons were distributed throughout layers II and III as well as within layers V and VI. Labeled non-pyramidal neurons were not reduced in the parietal cortex in AD compared to controls. In contrast, GluR2/3-positive pyramidal neurons were observed by 70% in layers II and III and 62% within layers V and VI of the parietal cortex of AD cases compared to controls. These latter data suggest that the parietal cortex GluR2/3 pyramidal neurons are particularly vulnerable to pathologic insult. We are now examining the extent to which AMPA receptors contribute to the degeneration of these cells.

272.5

IMMUNOLocalIZATION OF GLUTAMATE AND GLUTAMATE RECEPTORS IN THALAMOCORTICAL, THALAMOSTRIATAL AND CORCORTICOSTRIAL SYSTEMS IN TURTLES


Although thalamocortical, thalamostriatal and corticostriatal projection systems are present in turtles, it is uncertain if these systems utilize glutamate as a neurotransmitter, as has been shown to be the case in mammals. Immunohistochemical localization of glutamate and three of the four major glutamate receptors (GluR2/3 and GluR4) were used to address this issue. Glutamate immunoreactivity was found to be intense in all essential all neurons of the dorsomedial and dorsolateral thalamus, which appear to correspond to the mammalian intralaminar thalamic nuclei. Intense glutamate immunoreactivity was also observed in neurons of the nucleus reuniens, and less intense labelling was noted throughout the telencephalon, the pallial cell groups receiving thalamic input (i.e. the dorsal cortex, pallial thickening and the dorsal ventricular ridge) were rich in glutamatergic neurons. Additionally, these regions were pervasively rich in neurons possessing GluR2/3 subunits and a small subpopulation were rich in GluR4 subunits. A similar distribution of GluR2/3 and GluR4 subunits was found in the stratal part of the basal ganglia, and the pallidal region was specifically rich in large neurons possessing GluR4 subunits. These results indicate that the thalamostriatal, thalamocortical and corticostriatal projection systems of turtles are glutamatergic. Supported by NS-19620, EY-05298 (A.R.).

272.7

N-METHYL-D-ASPARTATE RECEPTOR (NMMPRI) SPlice VARIANTS DIFFER IN SELECTED REGIONS OF THE 3' AND 5' TERMINALS

M. Fotaki and R. P. Fotaki. Department of Pharmacy, Cornell University Medical College, New York, NY 10021.

The NMDA receptor plays an important role in excitatory neurotransmission and neuronal plasticity, and is thought to be involved in the mediation of adult neuronal plasticity. Pharmacologically distinct (homomeric) receptors are expressed from the eight cloned NMDA receptor subunits (Glur1-4, 6a,b, and 7). GluR1, GluR2, and GluR4 were used to address this issue. Glutamate immunoreactivity was found to be intense in all essential all neurons of the dorsomedial and dorsolateral thalamus, which appear to correspond to the mammalian intralaminar thalamic nuclei. Intense glutamate immunoreactivity was also observed in neurons of the nucleus reuniens, and less intense labelling was noted throughout the telencephalon, the pallial cell groups receiving thalamic input (i.e. the dorsal cortex, pallial thickening and the dorsal ventricular ridge) were rich in glutamatergic neurons. Additionally, these regions were pervasively rich in neurons possessing GluR2/3 subunits and a small subpopulation were rich in GluR4 subunits. A similar distribution of GluR2/3 and GluR4 subunits was found in the stratal part of the basal ganglia, and the pallidal region was specifically rich in large neurons possessing GluR4 subunits. These results indicate that the thalamostriatal, thalamocortical and corticostriatal projection systems of turtles are glutamatergic. Supported by NS-19620, EY-05298 (A.R.).

272.8

IN SITU HYBRIDIZATION STUDIES OF THE DISTRIBUTION OF A CPP-BINDING PROTEIN IN RAT BRAIN.


A complex of proteins that has recognition sites for NMDA receptor ligands was isolated from rat brain synapic membranes (Kumar et al., J. Biol. Chem. 264, 23734-23739, 1989). With antibodies to three of the proteins, a 70 kDa CPP-binding protein (GBP), a 63 kDa CPP-binding protein (CPB), and a 60 kDa CPP-binding protein (CPPBP), we were able to demonstrate the presence of these three proteins in the complex purified from synaptosomal membranes. The antibodies raised against the CPP-binding protein protein labeled a 80-93 kDa protein in synaptic membranes and were used to screen hippocampal cDNA libraries and to clone a 3.8 kb cDNA insert (Kumar et al., this meeting). An 1.4 kb PstI fragment from the insert was used to probe an antisense cRNA probe used in the in situ hybridization studies. The probes were labeled with [35S]CTP. The results of the in situ hybridization studies indicated that the expression of this protein was high in discrete regions of the hippocampus and was particularly high in the dentate gyrus, CA1, CA3, and CA4. In the hippocampus, the expression of the protein was highest in the CA1 and CA3 regions and was low in the CA4. The expression of the protein was also high in the entorhinal cortex. The expression of the protein was low in the dentate gyrus, CA1, CA3, and CA4. Our results illustrate the different regions of the hippocampus that are sensitive to drugs that affect the synaptic expression of the protein.
T72.9

NMDA antagonists, e.g. MK-801, have been investigated for their therapeutic potential in brain ischemia and other neuropsychi- atric diseases. However, the clinical potential of these agents may be limited by the observation that these NMDA antagonists cause neuronal injury or reversible neuronal swelling. This damage is manifest as a vacuolization in neurons of the cingulate and retrosplenic cortices as well as induction of the 70 kDa heat shock protein (HSP-70) in neurons. Memantine (1-amino-3,5-dimethyladamantan-1-ol) has been used clinically for the treatment of Parkinson's disease. It has been reported recently that memantine has an antagonistic effect at the NMDA type of glutamate receptor. Although numerous studies have reported the NMDA antagonistic properties of memantine, there are no data on neurotoxic effects of memantine in vivo. To investigate the effect of memantine in the CNS, the experiments were designed to examine the neurotoxic potential of memantine using the induction of HSP-70 as a marker of neuronal damage. Memantine (10-75 mg/kg, i.p.) was injected into male SD rats. The immunohistochemistry of HSP-70 was studied 24 hr after administration of memantine. The administration of memantine produced the HSP-70 in the primary cingulate and retrosplenic cortex of rat brain, similar with other NMDA antagonists. This result suggest that memantine has antagonistic effect at memantine receptor in vivo and raise the possibility that memantine may cause neuronal injury.

T72.11
SYNAPTIC AND SUBSYNSAPTIC DISTRIBUTION OF GLUTAMATE RECEPTOR SUBUNITS IN CEREBRAL CORTEX. Weinberg R.J.1,* Pogliatllo A., Westholm B.J.1 and Khairatia V.A.1 Dept. Cell Biology, UNC, Chapel Hill, NC 27599;1 Lab of Neurochemistry, NINDS, Bethesda, MD 20892.

I.M. immunocytochemistry suggests that glutamate receptors concentrate at synapses. This conclusion is generally supported also by EM studies, though non-synaptic staining has also been reported. To determine how exactly the location of these receptors in a postsynaptic cell, we used a new postembedding gold technique to use labeled glutamate receptor subunits in rat S-1. Analysis of gold particles coding for AMPA receptor subunits GluR1 A, 2, 3 and NMDAR1 revealed that labeling concentrated over active zones, with maximal density over the synaptic cleft and postsynaptic membrane. Significant labeling was also found in presynaptic terminals, especially for NMDA receptors. Subunits were also found within dendritic cytoplasm, concentrating ~20 nm inside the postsynaptic membrane. Distribution of all four receptor subunits across the synaptic cleft was similar; however, subunits differed in their tangential distribution along the active zone: GluR1 and NMDAR1 concentrated in central parts of the active zone, whereas GluR2 was denser at its periphery. These results demonstrate that Glu receptors subunits are mainly at synapses. Subsynaptic labeling may represent a reserve pool of receptor proteins. We hypothesize that rapid translocation of receptor from this pool into the synaptic membrane may be involved in certain types of synaptic plasticity. Supported by NIH # NS-29879 (to RWJ)

T72.12
AMP A AND NMDA RECEPTOR SUBUNITS UNDERLYING FINER CALIBER PRIMARY AFFERENTS. Pogliatllo A., Westholm B.J.1 and Rustioni A.1 Dept. of Cell Biology & Anatomay, UNC, Chapel Hill, NC 27599;1 Lab of Neurochemistry, NINDS, Bethesda, MD 20892.

Glutamate is the major neurotransmitter in primary afferents terminating in the dorsal horn of the spinal cord. Different classes of primary afferents may be presynaptic to different receptor types. We report initial results of an EM study of the distribution of these receptors, using a new postembedding immunogold technique. Rats were perfused with mixed aldehydes; sections, postfixed in tannic acid (rather than osmic acid) and treated with iodium tetrabromide and phentolamine, were processed as described previously (Pogliatllo et al., 1995) using antibodies for the AMPA subunits GluR1, GluR2/3 and NMDAR1. The distribution of gold particles coding for these antigens over different types of terminals was studied. Counts from all terminals in lamina II identifiable on morphological grounds as primary afferent terminals showed that the large majority of particles were in a region between 30 nm outside, and 40 nm inside the postsynaptic membrane. Particle counts coding for GluR2/3 and GluR2/3 were almost exclusively associated with the postsynaptic membrane; particles for NMDAR1 were also present more than 30 nm presynaptic to the active zone. Preliminary results indicate that primary afferent terminals of different morphologies, likely to exhibit differing functional properties, contact the GluR1, GluR2/3, and NMDAR1 in different subunits. Terminations likely to release both glutamate and neuropeptides were more likely to be presynaptic to NMDA receptors.

T72.13
CONFOCAL IMAGING OF A GLUTAMATE RECEPTOR SUBUNIT ON LIVING HIPPOCAMPAL NEURONS S.A. Richardson1, A.J. Irving2, F. Michaelis3, E. Molnar4, J.McIntyre5, W.W. Birmingham6, T.F. Rustioni7 and J.L. Phillips7. 1Dept. of Anatomy, Medical School, University of Bristol, Bristol BS8 1TD, U.K. 2Dept of Pharmacology and Biochemistry, 3University of Birmingham, Birmingham B15 2TT, U.K. 4MRC Analytical Neuropharmacology Unit, University of Oxford, Oxford OX1 3PH, U.K.

In order to observe glutamate receptor subunits in a living system, well-characterized polyclonal antibodies directed against the extracellular N-terminus of the AMPA receptor subunit GluR1 were applied to hippocampal neurons in culture. GluR1 immunoreactivity was visualized by the addition of fluorescent secondary antibody and by confocal laser-scanning microscopy (BioRad MRC 600 U/V). On the soma and dendrites of mature neocortical neurons, GluR1 immunoreactivity was associated with that of synaptophysin, although some extrasympathetic labeling was also apparent. Using a technique that enables discrimination between membrane-bound and intracellular immunoreactivity, cytosolic GluR1 immunofluorescence was present predominantly in the soma of hippocampal neurons, although some intracellular immunoreactivity was also present in primary dendritic regions. In addition, a few neurons that did not express GluR1 on the plasma-membrane displayed considerable cytosolic GluR1 immunoreactivity. These results demonstrate the viability of differentiating between populations of surface and intracellular receptor subunits. Supported by the MRC and Wellcome Trust.

T72.14
EXPRESSION OF THE NMDAR1 GLUTAMATE RECEPTOR SUBUNIT IN COS-7 CELLS. J.E. Rodie1, E.H. Franklin, T.J. Murray and M. Leid. College of Pharmacy, Oregon State University Corvallis, OR 97731.

COS-7 cells were transiently transfected with the rat NMDAR1 (NR11) subunit and the pharmacologic properties of homeric NR11 subunit recombinant receptors characterized using radioligand binding techniques. In addition, transient transfection of NR1 using electroporation and calcium phosphate methods were compared. A 3.5 kb HindIII - Stul fragment containing the NR1-1a coding and flanking sequences was excised from pN6(a) (gift from S.Nakaihisa) and inserted into a pcDNA3 plasmid pT1 (Leid et al., Cell, 68:377, 1992) to yield the cysrectic expression vector NR1. COS cells were cultured in Dulbecco's modified Eagle's medium and plated to a density of 6x104 cells/10cm plate one day prior to transfection. Cells were transiently transfected with 10 pg NR1 using a BTX ECM600 electroporation system or by calcium phosphate precipitation. PCR of the transiently transfected COS cells showed specific binding of the glycine site antagonist [3H]5,7-dichlorokynurenine acid (20 nM) 18 hours posttransfection. Expression of NR1 as demonstrated by specific binding of [3H]5,7-dichlorokynurenine acid, was enhanced ten-fold (1.8 pmol/mg protein) in COS cells transiently transfected by electroporation relative to those transfected by calcium phosphate. This work was supported by an NIH grant to TFM (DA 07218).
GLUTAMATE RECEPTOR SUBTYPES ON DISOCIATED MAGNOCELLULAR BASAL FOREBRAIN NEURONS IN CULTURE. D.J. Weeks,* T.G.J. Allin,* A.J. Sim and D.A. Brown* Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK.

Glutamate is the dominant excitatory neurotransmitter in the mammalian CNS. We have attempted to identify subtypes of glutamate receptor present on magnocellular cholinergic cells from rat basal forebrain nuclei (BFN). BFN neurons were cultured from 12-14 day-old Sprague-Dawley rats (Allin, Sim & Brown (1991). Physiol. Behav. 48, 115-116.) After 5 days to 3 weeks in culture, magnocellular cells were voltage-clamped using the whole-cell variant of the patch-clamp technique. Responses to glutamate receptor agonists were studied by pressure-ejection from a glass micropipette or bath-application.

Pressure-ejection of N-methyl-D-aspartate (NMDA) elicited an inward current with two components; one rapidly desensitizing and one sustained. Both components were sensitive to Ni(II) magnesium and to 2-amino-5-phosphono-pentanoate (AP5). Pressure-ejection of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) elicited an inward current with rapidly desensitizing and sustained components. Both components were antagonised by kynurenic acid and by 6-cyano-7-nitroquininal-2,3-diones (CNQX). Bath-application of AP5 produced a large steady-state current with an EC50 of approximately 10M. Bath-application of the benzenosulphonic cyclohexylidene glyptotid potentiated AMPA-induced current whereas the lecithin cannovalin A had little effect. Pressure-ejection of the non-NMDA agonists kainate and domoate elicited a sustained inward current, which was sensitive to both kynurenic acid and CNQX. Responses to pressure-ejection of domoate were reduced, but not eliminated, by bath-application of AMPA. These data indicate the presence of an NMDA-type, an AMPA-type and possibly a kainate-type receptor on these cells.

Supported by Eisai Research Laboratories and the MRC.


Developmental profiles of NMDA R1, R2A, and R2B subunits were determined in rat CNS. Quantitative immunoblots were used to determine protein levels. In all brain areas measured, levels of NR1 subunit increased 2.5-4.5 fold during the first three weeks after birth. There were large differences in the amounts (pmol/mg) of NR1 subunit expressed (2.5, HP; 1.3, HC; 1.0, OB; 0.85, MB; 0.41, CB). For NR2A, low levels were expressed in newborns followed by a progressive increase of this subunit from P7 to P22 at which time adult levels were reached. The ontogenetic profiles of NR1 and NR2A were similar to each other and correspond with mRNA expression studies. Furthermore, the absolute numbers obtained were close to those reported for 3HMK-B501 binding site densities. In contrast, ontogenic profiles of NR2B subunit were distinct in all brain regions. Typically, NR2B was expressed at relative high levels at early ages (P2-P4) which sometimes increased modestly over the next several days and then decreased. Peak densities of NR2B subunit in various brain regions were attained at different times (P4, CB; P7, HC, P11, MB; P16, HP). In OB, the levels of NR2B subunit were almost constant over the entire period tested. In CB, NR2B declined after P7 to undetectable levels at P22. These data provide a rationale to explain functional changes in NMDA receptor properties in different brain regions during development. Supported by NS28130, AG09973, AG09884.

REGULATION OF GABA_A ø1 SUBUNITS, NEURONAL GROWTH AND SURVIVAL BY GABA_A RECEPTOR LIGANDS IN EMBRYONIC RAT BRAINSTEM CULTURES. L.Liu,* A.J. Moore* and J.M. Lander* Dept. of Cell Biology and Anat., Dept. of Psychiatry, Univ. of N. Carolina, Chapel Hill, NC 27599.

Previous studies have indicated the presence of functional a, b, y and 6 subunits of GABA_A receptors in embryonic rat brainstem cultures. GABA_A or GABA_B antagonist bicuculline differentially regulate the survival of brainstem monoamine and GABA neurons (Liu et al., 1994). In the current study, dissociated cultures were prepared from embryonic day 14 (E14) brainstem and cultured in BME + 10% NCSerum for 1 day in vitro (DIV), then treated for 48 hrs with 10 M GABA and 10 M bicuculline or the GABA_A antagonist bicuculline in serum-free medium. Effects of GABAergic agents on relative levels of a1 protein were determined by quantitative immunochemical assays and absolute amount of a1 mRNA transcripts were quantified by competitive PCR. Results indicated that expression of a1 protein and mRNA were up-regulated by both bicuculline and dieldrin. Effects on survival of neurons were quantified by counts of immunoreactive cells. GABA_A promoted survival of 5HT and TH neurons. Dieldrin blocked this effect, and by itself had inhibitory effects on survival of these cells and stimulatory effects on survival of GABA_A neurons. Cell size, shape and complexity of neurite outgrowth by 5HT and GABA_A neurons were analyzed by computer-assisted morphometry. GABA_A, bicuculline and dieldrin also had differential effects on neurite outgrowth by these neurons depending on their neurotransmitter phenotype. These results suggest that GABA_A agonists or antagonists may be used to regulate the expression of GABA_A receptor subtypes in developing neurons and may regulate expression of its own receptors during brain development. GABA_B antagonists, such as organochlorine pesticides may interfere with this regulation. Supported by NIEHS grant to JL. The authors thank Dennis Grayson for providing a1 internal standards for competitive PCR.
T23.3 GABA CURRENTS IN DEVELOPING MEDIAL SEPTUM/ DIAGONAL BAND (MS/DB) NEURONS. S.H. Hsu*, L. West, and F. Deisseroth, Departments of Pharmacology & Toxicology, The University of Texas at Austin, Austin, TX 78712.

The GABA receptor is a ligand-gated channel which plays a prominent role in the development of the central nervous system. During postnatal development, the expression of GABA receptor subunits undergoes major changes (Laurie et al., J. Neurosci. 12:4151, 1992) and receptor function may shift from mediating excitation to inhibition (Chaiara et al., J. Neurosci. 15:2639, 1995). The present study examined changes in GABA currents in cultured dissociated neurons from MS/DB of postnatal male and female Sprague Dawley rats. Two age groups of rats were examined (postnatal day 8 and 16). GABA (0.3-300 μM response) waves were concentration-dependent; maximum response was obtained with 10 μM GABA (0.3-300 μM response) in cultures of neurons derived from the postnatal day 16 group. The Hill coefficient was 1.6±0.16 (n=5, p<0.05). The Hill coefficient of 1.6±0.16 (n=5, p<0.05) did not change for juveniles vs. pups, respectively. These data suggest that there are significant changes in GABA receptor function in MS/DB neurons during early development postnatal day 8 and 16.

T23.4 DEVELOPMENTAL INDUCTION OF GABA RECEPTOR α1-SUBUNIT POLYPEPTIDES IN CHICK EMBRYO CORTEX AND DERIVED NEURONS IN CULTURE. J.R. Miranda* and F.M. Barnes, Department of Neurosciences, Baylor College of Medicine, Houston, TX 77030.

In order to produce high-titer antibodies which are capable of recognizing native GABA receptor subunits, we have produced fusion proteins containing selective receptor sequences in the intracellular loop of the subunits. The fusion protein containing the α1 stretch of the α1 subunit was expressed in a bacterial strain harboring the α1 subunit cDNA and the affinity purified fusion protein was used to raise a high titer antibody in rabbits. The antibody binds to GABA receptor subunit containing synaptic terminals of chick embryos 16 days in ovo. The threshold for antibody saturation, 1 μl of crude antiserum was able to immunoprecipitate 61% of [3H]-flunitrazepam binding. The antiserum is also selective because no cross reaction was detected on blot analysis of 16-437 fusion proteins. Western blots utilizing a 1:1000 dilution of the R4 immune serum showed a reaction with a single 50-51 kDa polypeptide from chick embryo cortex. This protein was not detected in heart, liver or skeletal muscle. Similar analysis on immunoblots revealed an increase in the expression of the GABA receptor α1-polyepitope in chick cortical neurons from day 2 to day 6 in culture and in chick cerebral cortex from embryonic to adult stages. In both cases this pattern of developmental expression was similar to that previously reported for the major postnatal ligand binding sites of GABA receptors.

Supported by DK71436, MH74715, GM14156, and NS11533 from NIH.

T23.5 A RHÔ-LIKE GABA RECEPTOR SUBUNIT IS TRANSPARENTLY EXPRESSED IN NEONATAL RAT BRAIN. M. Madnic, E. Cherubini*, Biophys Lab, Int. Sch. Adv. Studies (SISSA), 34014 Trieste, Italy.

A novel bicuculline and baclofen insensitive chloride mediated response has been described in neonatal hippocampal cells (Strata&Cherubini, J. Physiol. 480:492-503, 1994). This response clearly resembles the one present in the retina and supposed to be mediated by GABA receptors. It is conceivable that this novel receptor channel is the homologous engineered protein assembled from the α1 subunit. To see whether a rhô-like GABA receptor subunit is present in the rat brain, in situ hybridization on brain sections from neonatal and adult rats were performed. RHô1 and RHô2 GABA receptor subunit specific antisense oligonucleotide probes as well as another probe that revealed both RHô1 and RHô2 subunits (RHô1+RHô2) have been used. The rhô1 probe was homologous to the 1325-1372 nucleotide sequence of the rhô1 gene (Cutting et al., Proc. Natl Acad. Sci. USA, 88:2675, 1991) and the rhô2 probe to the 1290-1331 nucleotide sequence of the rhô2 gene (Cutting et al., Genetics, 12,804, 1992). The rhô1+rhô2 probe was homologous to the nucleotide sequence of the rhô1 gene (1130-1174) and nucleotide sequence of the rhô2 gene (1942-1989). As a positive control a beta subunit specific antisense probe was used. Control group were sense oligonucleotide probes. The same probes were utilized to hybridize sections of the rat retina. The autoradiographic signals obtained with ρhô1 and ρhô2 probes were low in the neonatal as well as in the adult brain. However signal obtained with ρhô1+rhô2 probe was surprisingly strong in the neonatal brain sections, especially in the forebrain or in the hippocampus. In the retina high signals with all three ρc probes were obtained. All the brain sections hybridized with ρhô1+rhô2 probe were present in the cerebellum but comparing to neonatal brain was lower. These results strongly suggest that a novel rhô-like subunit is expressed in the neonatal brain during a critical period of postnatal development.


Developmental modulation of GABA receptor function, via administration of diazepam (DZ) to the pregnant dam, alters behavioral and GABA receptor responsiveness to stress in the adult rat. Recent studies have also shown that the molecular composition of the GABA receptor is linked to pharmacologic responsiveness of the receptor. Functional exposure to acute environmental challenges changes mRNAs levels of specific receptor subunits. In this study, we evaluate the effect of exposure to DZ (2.5 mg/kg) over gestational days 14-20 on basal and stress-induced mRNAs levels for α1, γ2 and GABAC receptor subunits in the cerebral cortex, hippocampus, and hypothalamus of adult (60-70 days) male rats. The results are compared to those from animals exposed over the same period to vehicle (4% ethanol glycine, 10% ethanol) or co-exposed to DZ and the antagonist flumazenil (10 mg/kg). Early exposure led to a significant 15% increase in the basal level of mRNAs for α1, γ2 and GABAC receptors, compared to controls. The increase was blocked when DZ was prevented by co-exposure to flumazenil. There was no change in α1 mRNA in any other region, and γ2 mRNA was unaffected in all regions examined. In early DZ exposure interferes with environment-specific behavior, we have examined the effect of this challenge on mRNAs for the two subunits. In naive adult male rats, cortical mRNA for α1 was highly expressed following the period of testing in either a familiar or novel environment. The impact of early DZ exposure on this response is being evaluated. Supported by Grant No. DA 00708.

T23.7 DIFFERENTIAL INTERACTION BETWEEN NEUROACTIVE STEROIDS AND DIAZEPAM AT THE GABA RECEPTOR IN RAT FETAL VS ADULT BRAIN. C.K. Kellogg* and G.L. Pleger, Dept. of Psychology, Univ. of Rochester, Rochester, New York, 14627.

Specific neuroactive steroids elicit positive modulatory effects on the GABA receptor. This observation, coupled with our observations that prenatal exposure to diazepam (DZ) alters neural and behavioral stress responses that emerge over adolescent development under the influence of gonadal steroids, led us to hypothesize that neuroactive steroids may exert organizational influences during development via the GABA receptor. Early exposure to DZ could alter this influence. Measuring GABA-stimulated [3H]chloride influx in synaptoneurosomes prepared from the cerebral cortex of adult males and fetal forebrain at 20 days gestation, we evaluated the interaction between DZ and neuroactive steroids that affect the GABA receptor and that could be present in fetal tissue during gestation. The 5α3β-progestrone metabolite, pregnanolone (P, 500 nM), increased the potency (decreased the EC50) of GABA in both adult male and mixed-sex fetal tissue by 38-48%. DZ also increased the potency a comparable amount in adults, but the effect was much more pronounced in fetal tissue (a 77% decrease). The in vitro addition of both DZ and P had an additive effect in the adult but in fetal tissue, the potent effect of DZ masked an effect of P. The testosterone metabolite, androsterone (2.3 μM), also decreased the EC50 in adult and fetal tissue (separated by sex), but the effect was more pronounced in fetal tissue, particularly in female forebrain. Supported by Grant No. DA 00708.


In this work we study the effects of exposure frequency of CI channel of the GABA-BZD receptor complex on the sexual differentiation of maternal behavior (MB) and its correlation with morphological and behavioral alterations. Dizygotic (DZ) and monozygotic (MZ) rats is as follows: MB is a sexually dimorphic behavior; MZ is under hormonal control and tonic androgenic influences (T); the embryonic medium (EM) is a sexually dimorphic neural system; 4) GABA is present in about 90% of the accessory olfactory bulb (AOb) granule populations; 5) the genetic substratum sex-linked sexual differentiation prorogates a female morphogenetic pathway of the AOb (olfactory bulb) in female, modulating the olfactory bulb number in the male rat and facilitates the induction of MB in virgin females when adult; 6) DZ increases the opening frequency of the CI channel of the GABA receptor, which in male rats, induces the levels of progesterone (P) decrease. We hypothesize that treatment during developmental periods with drugs acting in an opposite way on the GABA-BZD CI channel (DZ/PC) would cause opposite behavioral, morphological and/or androgenic effects. Fifty rat pups of the Wistar strain were randomly divided in the day of birth into four groups: a) untreated males (M) N=15; b) 1.5 mg/kg of DZ injected males (DZ/male) N=15; c) untreated females (F) N=15 and d) 0.5 mg/kg PC injected females (PC/female) N=15. MB tests were performed at 20 days of age. EMBR Software was used to register MB, Blood samples were collected before transcardiac perfusion, AOB and histologically processed. DZ treatment significantly increased the frequency of copulatory patterns in relation to both males and PIC females: Nest making, grooming, licking and physical contact and percentage of animals reaching sexual maturity (80% of days of complete retrieval). DZ males did not differ from untreated males in any behavioral pattern, except in licking. Our results indicate that CI channel is involved in the sexual diversification of the VNS. The work was supported by DICYCT PM/79-027 (IT) and PIBA 03291-320-3-3 (IT).
723.9

LOSS OF Nk-A-ATPase α-3 ISOFORM mRNA EXPRESSION IN THE INTRAPONTONTAL AREAS OF AGING HIPPOCAMPUS. N. S. Chauhan* and G. L. Sigel. Cell Biology and Molecular Neurosciences, Department of Neurology, VA/Loyola University Chicago, I., Hines, IL 60141.

Age-related changes in the expression of Nk-A-ATPase α-3 and α-2 isoforms mRNA in young (3 months) and old (24 month) Fischer 344 male rats were analyzed. In situ hybridization was performed on 5-μm thick paraffin sections of parietal cortex and brains, and sections with 35S-labeled riboprobes, Section hybridization were performed at 42°C, treated with RNase A, washed, and exposed to NTB-2 Kodak emulsion for 4 weeks at 4°C. After development and counterstaining with hematoxylin and eosin, sections were mounted, dried and stabilized with the use of the BioQuant Image Analyser. Sense controls showed no significant labeling. Total grains were counted in 10 squares of 250 μm² in each of three sections. A significant decrease in signal was observed throughout the whole brain. The changes in signal intensity were consistent with the aging process. The results suggest that the decrease in Nk-A-ATPase α-3 mRNA expression may be related to the aging process.

723.10


GABAergic gated chloride channels have been found to be present in all retinal neurons, mediating a wide variety of responses belonging to the classically defined "GABAa" subtype. The responses mediated by these receptors are characterized by a rapid, non-voltage-dependent desensitization by bicuculline. Studies by other investigators have demonstrated the presence of multiple consensus sites for phosphorylation by a variety of protein kinases. The effects of these kinases on GABAAergic responses were investigated in cultures of retinal ganglion cells using fast flow perfusion and conventional cell-attached recording techniques. In agreement with (Veraki and Yeh 1994), we find that the application of Vinculinocative Intestinal Peptide (1μM), which is known activate adenylate cyclase, transiently potentiates GABA evoked responses in ganglion and bipolar cells. Either Phorbol-12-myristate-13-acetate (10μM) or Phorbol-12,13-dibutyrate (1μM), in the intracellular pipette solution did not alter the rate at which GABA responses rundown. In order to examine the effect of PKI mediated phosphorylation we perfused extracellularly with a cell permeant cGMP analogue known to activate PKG. One minute application of either cGMP-1 mM resulted in a 15% decline in the GABA evoked response as compared to 2.6% for controls. In sum, ganglion cells are able to fine tune their response to GABA by selective activation of different protein kinases. Supported by NIH grant EY-10254, and ALCON Laboratories.

723.11


Increasing reports on intracellular regulation of the GABAa receptor show possible involvement of phosphorylation/dephosphorylation of the receptor. Consensus sequences for protein kinase(s) are inferred from some substrates DNA sequences. Electrophysiological studies reveal that desensitization and 'channel blockers' of GABAa receptors may be related to their state of phosphorylation. In this study, alterations in GABAa receptors caused by phosphorylation were visualized using receptor autoradiography. Frozen rat brain slices were treated with 50 μM Mg-ATP at physiologically relevant conditions in 50 mM Tris-phosphate, 200 μM NaCl and 1 mM EDTA. Treated slices were then incubated in the phosphate buffer with 3 H]-TBPS. Major alterations were observed in the cerebellum. Binding was decreased in the granule cell layer (30%), with a significant increase (26%) in the molecular layer. Since the cerebellum is localized to the granule cell layer, the observed increase may be related to this subunit. Moreover, since the mean changes in binding were in either direction, the effect of phosphorylation may be region specific. Although this is an indirect assay for receptor phosphorylation, our method detected region-specific alterations that biochemical studies can not detect. It may be a powerful and versatile tool for studies of receptor phosphorylation for other receptors as well as GABAa receptors. (Supported by NIDA-04880)

723.12

PHOSPHATE INHIBITORS SHORTEN IPSC DECAY BY ALTERING GABA CHANNEL KINETICS. M. Y. Jones* and G. L. Wantanabe. Dept. of Pharmacology, Rush University Medical Center, Chicago, Ill. 60612.

Phosphorylation and dephosphorylation are potentially important mechanisms for regulating the shape of inhibitory post-synaptic currents (IPSCs), but have usually been studied with applications of low GABA concentrations. The synaptic GABA transient, however, is thought to be quite high and brief. Therefore, we studied effects of phosphorylation inhibitors on the decay of inhibitory postsynaptic currents (IPSCs) in patch clamp experiments. Whole-cell recordings were held for 200 seconds before pulling patches when phosphate inhibitors or ATP were present in the patch pipette. All current decay rates were fit with two exponentials, but for simplicity time constants are expressed as an average time constant: T(1) = (t(1) + t(2)) x 1/2 [t(1) x t(2)]. ATP(5) (5 mM) significantly (p < 0.05) shortened IPSC decay from 43 ± 2 ms (n = 3) to 11 ± 1 ms (n = 5). ATP(10) (10 mM) also significantly shortened the decay rate from 43 ± 2 ms (n = 5) to 40 ± 1 ms (n = 3). ATP(100) (100 mM) further shortened the decay rate to 39 ± 2 ms (n = 3). These results suggest that a decrease in decay rate requires a decrease in decay rate.

723.13

NOREPINEPHRINE AND CAMP ENHANCE DENTATE GRANULE CELL GABAERGIC RECEPTOR CURRENTS. Jaideep Kapur* and Robert L. Macdonald. Dept of Neurology, University of Michigan, Ann Arbor, MI 48104.

There is an association of noradrenergic and GABAergic terminals on the penrykaya and dendrites of dentate granule cells. The effect of noradrenergic stimulation on GABAa receptor (GABAa) currents in dentate granule cells is not known.

Dentate granule cells were acutely isolated and GABAa currents were recorded using conventional whole cell patch clamp methods. Drugs were applied as rapid application system

500 μM or 1 mM noradrenalin and the selective β-adrenergic agonist, isoprenaline enhanced GABAa currents elicited by 30 μM or 1 mM GABA in 12/16 granule cell cultures also consistently enhanced dentate granule cell GABAa currents elicited by 30 μM or 1 mM GABA. The effect of CAMP on GABAa concentration response relationship was studied and the data were fitted to a sigmoid dose response curve. Maximal current was enhanced in all cells (40% ± 12%, N = 4, p < 0.04). The EC50 shifted to the left in 2 of 4 cells, from 95 μM to 20 μM (p < 0.28). We wished to test if noradrenalin and CAMP could affect GABAa currents in a dose-dependent manner. CAMP concentration response curves were obtained in dentate granule cell populations with intracellular recording solution containing no ATP (Maximal current: I50 = 174 pA, N = 8, p < 0.05). CAMP concentration response curves were obtained in dentate granule cell populations with intracellular recording solution containing no ATP (Maximal current: I50 = 174 pA, N = 8, p < 0.05). ATP (1 μM) increased GABAa currents elicited by 30 μM or 1 mM GABA by 20 μM, p < 0.04. Noradrenalin, isoprenaline, norepinephrine, and ATP consistently enhanced maximal dentate granule cell GABAa currents, and produced a left shift of GABAa concentration response curve.

723.14


Whole vertebrate GABA receptors have been shown to be regulated intracellularly by phosphorylation. little is known about the intracellular regulation of insect GABA receptor function. Toward this end, small diameter (~20 μm) neurons from the sixth abdominal ganglion of the American cockroach, Periplaneta americana, were enzymatically isolated. The effects of exogenous adenosine analogues were studied in the cockroach GABA receptor function.

When added to the intracellular medium, low ATP completely blocked GABA response rundown. ADP also slowed GABA response rundown, but responses stabilized at a level about half that of ATP. In the presence of PKA, ATP was only as efficacious as ADP in slowing rundown. PKA had no effect on the ability of ADP to slow rundown, suggesting that the co-phosphate of ADP is not involved in PKA-dependent phosphorylation of the GABA receptor. Guanine nucleotides had no demonstrable effect on GABA responses. These results suggest that in cockroach neurons, GABA receptor function is maintained intracellularly by adenosine nucleotides, not only by phosphorylation, but also by an interaction with a nucleotide recognition site unrelated to PKA-dependent phosphorylation.
GABA receptors exhibit various responses.

Many studies have demonstrated that phosphorylation modulates the function of ligand-gated ion channels; however, the effects of phosphorylation on the γ-aminobutyric acid type A receptor (GABA_A) remain controversial. In the present study, we examined the effects of CAM-dependent protein kinase (PKA) on the function of recombinant human GABA_A receptors expressed in a 129 mouse fibroblast cell line. GABA currents were recorded using conventional whole-cell patch-clamp methods with symmetrical chloride concentrations at holding potentials of -75 mV. Complete GABA dose-response curves were obtained for each condition examined. To reduce the variability often encountered when comparing populations of cells, a reimplantation protocol was used in which paired whole-cell recordings were obtained from individual cells. When a standard intracellular solution was used for both the initial and reimplantation cell recordings, GABA currents increased (100-500 μM) uniformly (50-120%) with minimal change in E_max (0.75-9.3 μM). In contrast, PKA catalytic subunit applied intracellularly on reimplantation enhanced peak current amplitudes when compared to the initial control implantation (50-120%, n=13), with no significant shift in GABA E_max (14.1 μM). This effect was associated with reduced desensitization of the GABA response. Heat-inactivated CaM-KII (n=12) did not alter the GABA responses. In addition, GABA-mediated inhibitory post-synaptic potentials in rat hippocampal CA1 neurons were enhanced by the subunit of CaM-KII, either by direct CaM-KII phosphorylation of the GABA_A receptor, and glycine receptors, or indirectly through phosphorylation of some membrane-bound regulatory proteins. This mechanism may contribute to long-term enhancement of inhibitory synaptic transmission. (Supported by NS-2652 and BNI-9203462).


Whole cell voltage and current clamps were employed to investigate the developmental changes of GABA, glutamate, and glycine receptors in cultured embryonic neurons (from 140) from rat hypothalamus. Each neuron studied from a few hours after plating responded to GABA (30 μM). The currents evoked by GABA increased by 5-fold within two weeks in culture. The time constants of the desensitization of GABA currents did not change during this period. In younger cultures, GABA currents were observed to increase and be potentiated by glutamate and glycine. This indicates that GABA currents in hypothalamic neurons may be regulated by the presence of other neurotransmitters.


Glycine (Gly) is an important inhibitory transmitter in the spinal cord and other regions of the CNS, and the modulatory influence of phosphorylation by protein kinase C (PKC) on the Gly receptor has been explored for oxytocin injected with either poly(A)+ mRNA isolated from neurons or synthetic mRNA, little is known about the influence of PKC on the native Gly receptor protein of intact cells. Therefore, we examined the effect of phosphorylation on Gly Aktivated currents, GIC, of hypothalamic neurons freshly isolated from the brains of young mice. GIC was recorded with the nystatin perforated patch technique in order to minimize perturbations of the cytoplasmic milieu that occurs with conventional whole cell recording. Bath application of 1 μM PDBu consistently depressed GIC; for 7-10 alone, separate peaks, peak Gly declined to 36.6 ± 15.3 % of control. Although the time course of this effect was variable, peak suppression occurred within 2-5 min after PDBu. For some neurons, the suppression of GIC persisted for 30 min even in the continued presence of PDBu. These results suggest that the Gly receptor can be phosphorylated by PKC. Thus, neurotransmitters affecting the activity of PKC could profoundly alter the efficacy of Gly as an inhibitory neurotransmitter in the CNS. NIAAA AA08025 and NIH NS31040.

REGULATION OF γ-AMINOBUTYRIC ACID TYPE A (GABA_A) RECEPTORS BY PROTEIN PHOSPHORYLATION. R. J. McDonald, G. H. Siddall, J. B. Sato, C. M. Connolly, A. Arnao, T. G. Smart, and S. J. Moss.

MRC LMB, University College London, WC1E 6BT and 1 School of Pharmacy, London WC1N 1AX.

We have investigated the role of protein serine/threonine and tyrosine kinases in modulating the function of GABA_A receptors. All seven subunits are phosphorylated on a conserved serine/threonine kinase sequence (PKA, PKG, PKC and CamKII, the α, β and γ subunits also contain a second site that is phosphorylated by PKC). Both γ2 and γ2L subunits, (γ2 and γ2L) are phosphorylated by PKC and CamKII; the γ2L subunit having an extra recognition site for both kinases. Receptor phosphorylation by protein serine/threonine kinases reduces the response to applied GABA, the magnitude of this effect being dependent on subunit composition. The amino acid sequence YRGRK, phosphorylated by PKA is conserved in all GABA_A receptor subunits, and results in specific phosphorylation of the β1, γ2L and γ2 subunit. The results of this phosphorylation are enhanced by application of GABA.


Evidence for a novel γ-aminobutyric acid (GABA) response was found for glutaminergic excitatory post-synaptic potentials in the early embryonic chick nucleus tractus solitarius (NTS), using a multiple-site optical recording technique employing a fast voltage-sensitive dye. In various areas of the ventral central nervous system, it is known that release of GABA provides inhibitory modulation of excitatory post-synaptic potentials. Classically, two classes of GABA_A receptors, GABA_A and GABA_B have been identified. The GABA_B receptors are blocked by the competitive antagonist bicuculline, and the non-competitive blocker picrotoxin. The GABA_A receptors are specifically blocked by γ-hydroxyclofen. In addition, bicuculline- and 2-hydroxyethylammonium-sensitive GABA_B receptors have been described. The GABA response found in the present experiment was insensitive to GABA_A antagonists (picrotoxin, dieldrin, bicuculline, SR95531) and GABA_B antagonists (2-hydroxyclofen, phaclofen, CGP35346), but was stimulated by either muscimol or baclofen.

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GABA RECEPTORS V

THE GABA\(_{\beta}\) RECEPTOR \(\beta 4\) SUBUNIT IS AN EMBRYONIC ISOFORM IN THE CHICK CEREBRAL CORTEX. M.H. Jalilian Tehran\*a, B.J. Baumgartnerb, and E.M. Barnes, Jr., Dept. of Biochemistry, Baylor College of Medicine, Houston, TX 77030.

Because of the great diversity of GABA\(_{\beta}\) receptor subunits, selective antibodies are invaluable reagents for examining receptor polypeptide distribution and regulation. Since cultivation labeling of these subunits is not possible, an effort was made to use anti-\(\beta 4\) antibodies to probe for immunoreactivity with adult brain. Immunoreactivity was detected in the chick GABA\(_{\beta}\) receptor \(\beta 4\) subunit. The \(\beta 4\) subunit was cloned in cDNA libraries to determine its amino acid sequence. The amino acid sequence of the chick \(\beta 4\) subunit was compared to known\(\beta 4\) subunit sequences from other species, and it was found to be highly conserved across species.

This study was supported by DK17436, MH7715, GM14156, and NS153 from NIH.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION IV

CLONING AND CHARACTERIZATION OF THE RAT GALR1 GALANIN RECEPTOR FROM RIN 14B INSULINOMA CELLS E.M. Parker, D.G. Tzarrifell, H.P. Nowak, C.D. Mullleh, L. Bem, J. Wang and M.E. Goldstein, Dept. of Psychobiological Disorders, Bristol-Myers Squibb Co., Wallingford, CT.

Galanin is a ubiquitous neuropeptide that regulates a wide array of physiological processes via interaction with specific G protein-coupled receptors. A rat galanin receptor cDNA was cloned from the RIN14B insulinoma cell line. The isolated clone encodes a 346 amino acid G protein-coupled receptor that is 92% identical to the recently reported human GALR1 galanin receptor (125). Galanin binds to high and low affinity states of the rat GALR1 receptor in COS1 cell membranes (K\(_{D}=19\) PM, Bmax=0.47 pmol/mg protein, K\(_{D}=300\) PM, Bmax=1.63 pmol/mg protein). Galanin, N-terminal galanin fragments, and the putative galanin receptor antagonists galantide, C7, M35 and M40 bind with high affinity to the rat GALR1 receptor. In contrast, C-terminal galanin fragments do not bind to this receptor. Galanin inhibits basal and forskolin-stimulated cAMP formation in CHO cells expressing the rat GALR1 receptor via a pertussis toxin-sensitive G protein. The GALR1 receptor is expressed in rat spinal cord, small intestine, RIN14B insulinoma cells and several brain regions, particularly ventral hippocampus, amygdala, suprachiasmatic nucleus, hypothalamus, thalamus, lateral parabrachial nucleus and locus coeruleus. The atrophied rat embryo, the GALR1 receptor is expressed in brain, spinal cord, dorsal root ganglia and small intestine. Cloning of the rat GALR1 galanin receptor cDNA will permit many new experimental strategies to be applied to studies of the structure and function of galanin receptors.


Angiotensin II (AngII) receptor levels are high in neonates and decrease to adult levels as the animal matures. A larger proportion of this reduction is due to decreases in Type 2 (AT2) receptors rather than Type 1 (AT1) receptors. In the present study, AT1, AT2, and AT3 receptors were used to study the distribution of AT1 receptors in the developing rat brain and in peripheral tissues by immunohistochemical and immunoblot analysis. Rat brains at 4, 5, and 12 days of age were examined via immunohistochemical techniques and compared with previous results from adult rat brain. As with the mature animal, AT1 receptor immunoreactivity was seen in the locus coeruleus, the supracarotid nucleus and the paraventricular hypothalamic nucleus. AT2 receptor staining was also associated with the paramicell cell layer of the cerebellum, as was seen in the adult rat. Densitometric analysis from the adult AT1 receptor immunoreactivity in the neonatal rat pup was less dense in the hippocampus. Immunoblot analysis was performed to determine the expression of AT1, AT2 receptors in both neonatal and adult rat peripheral tissues. Interestingly, AT2-directed antibodies did not immunodetect proteins in the neonate or adult adrenal, a tissue which is known to exhibit AT1 binding activity. However, in the neonate a 66 kDa immunoreactive protein was detected in the anterior pituitary. In neonatal striatum, immunoreactive bands of 210, 66, and 50 kDa were observed, with the 66 kDa band being the most intense. In contrast, the major immunoreactive protein was approximately 50 kDa in adult cortex, striatum and kidney. Collectively, these results establish the localization of AT1 receptors in the developing rat brain, in addition to examining AT2 receptor expression in neonatal and adult peripheral tissues. These results further support the hypothesis of AT1 receptor heterogeneity. (Supported by NS47878 and NS06986)

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE SOMATOSTATIN RECEPTOR SSTR2 IN RAT BRAIN USING A SPECIFIC ANTI-PEPTIDE ANTIBODY. L. Dumont, Y.Z. Ou, A. Schoneveld, I. Manttila, G.S. Tannenbaum, and A. Beaudet. Mc Gill University, Montreal, Quebec, Canada, H3A 2B4 and University of Texas, Houston, TX 77025.

In the present study, we have used a rabbit polyclonal antibody generated against a unique sequence in the SSTR2 receptor to visualize the regional and cellular distribution of this receptor subtype in rat brain. Specificity of the antibody was demonstrated by Western blotting, and by the observed localization of SSTR2 receptors in COX-2 expressing cells transduced with the CDNAD encoding SSTR2, but not SSTR1 nor SSTR6 receptor subtypes. In rat brain sections, SSTR2-like immunoreactivity was mainly expressed within neocortical perikarya and dendrites distributed throughout layers II-III and V of the cerebral cortex, the lateral septum, caudate putamen, nucleus accumbens, central amygdaloid nucleus, medial habenula, locus coeruleus and the pyramidal cell layer of the CA1-CA2 field of the hippocampus. Dense pleating of SSTR2-immunoreactive axon-like fibers were also observed in the deep layers of the cortex, claustrum, basolateral nucleus of the amygdala, and superior colliculus. Only sparse, moderately labeled perikarya and fibers were apparent within the hypothalamus, namely in the preoptic area, periventricular nucleus and accumbens nucleus. The thalamus and cerebellum were both devoid of immunoreactivity. In the hippocampus, medial habenula and central amygdaloid nucleus, the immunoreactivity was found by electron microscopy to pervade the cytoplasm of perikarya, dendrites and dendritic spines. In the medioventral mesencephalon, a few axon terminals also displayed weak reaction suggesting a presynaptic localization of SSTR2 in this area. The present results provide the first demonstration of the regional and cellular distribution of SSTR2 receptor in rat brain, and suggest that this receptor subtype is involved in the transduction of both pre- and postsynaptic effects of somatostatin in the central nervous system.


An angiotensin type 2 (AT\(_2\)) receptor identical to other cloned AT\(_2\) receptors has been isolated from a murine neuroblastoma N1E-115 DNA library using homology-based polymerase chain reaction. When transfected into COS-1 cells, this clone displayed (i) AT\(_2\) pharmacology, (ii) GTP\(_7\)-inhibitory agonist binding, and (iii) augmentation of agonist binding by diltiazem. Previously, we had demonstrated that 115 cells possess two distinct subpopulations of AT\(_2\) receptors, defined as Peak 1 and Peak 3 receptors (Simmons and J. Neurochem. 52:1393, 1994). The pharmacological profile and distinctive ligand binding properties in the presence of GTP\(_7\)-diltiazem of the closed AT\(_2\) receptor are consistent with that of Peak 3 receptors. Moreover, antagonists raised against Peak 1 receptors failed to immunoreact with either Peak 3 or closed AT\(_2\) receptors. Collectively, these data suggest that the cloned AT\(_2\) receptor is identical to Peak 3 receptors and that an apparently novel AT\(_2\) receptor (in Peak 3) remains to be cloned.

It was previously shown for the AT\(_2\) receptor that mutating a specific lysine (Lys\(_{199}\)) residue to glutamine diminished its affinity for peptide ligands (Yamasaki et al., JBC 267:1462, 1992). An analogously mutated AT\(_2\) receptor (Lys\(_{199}^{1462}\)) when expressed in COS-1 cells, possessed greatly reduced binding for the agonist angiotensin II compared to wild type receptor. More extensive pharmacological analysis of the mutated receptor to both peptide and non-peptide ligands is currently in progress. The effects of the lysine mutation on the AT\(_2\) receptor suggests that despite the low homology between AT\(_2\) and AT\(_1\) receptors (only 34%), some communialties in the binding mechanism for angiotensin II exists between the two receptor subtypes. (Supported by NS23986 and MH45787)

Previously, we have demonstrated that murine neuroblastoma N1E-115 cells possess both Type 1 (AT1) and Type 2 (AT2) angiotensin receptors. Inducing differentiation of these cells by the addition of dimethyl sulfoxide (DMSO) results in up to a ten-fold increase in the expression of AT2 receptors. In order to evaluate the efficacy of antsense oligonucleotides in reducing expression of this angiotensin receptor subtype, either AT2 antisense or scrambled 15-mer oligonucleotides were administered concurrently with DMSO treatments. Following three 24 hr treatments of the D-SiO, the cells were harvested and AT2 receptor expression was determined by radioligand binding assays. At concentrations of either 50 nM or 500 nM, the AT2 antisense oligonucleotide effectively attenuated the upregulation of these receptors during differentiation by approximately 40% compared to cell cultures treated with scrambled oligonucleotides. These studies are the first demonstration that antsense oligonucleotides decrease in vitro the expression of the AT2 receptor subtype and therefore should prove to be an important new tool in studying function and regulation of this angiotensin receptor subtype. (Supported by NS23986 and MH43787)


The rat Zucker strain has a recessive mutation responsible for obesity in homozygous animals. Obese fa/fa rats present numerous abnormalities, in particular hyperinsulinemia, hypogonadism and an altered response to stress. In normal rats, AVP-R and oxytocin receptors (OT-R) are present in brain regions implicated in the regulation of these hormones, i.e. the hypothalamus, the hippocampus and the dorsal motor nucleus of the vagus nerve. This suggests 1) that AVP and OT may participate to this regulation and 2) that the expression of AVP-R and OT-R are modulated by these hypothalamic and hippocampal signals. To test these hypothesis, brain slices of obese and lean rats were labelled using two iodinated ligands specific for V1A- and OT-R and for OT-R respectively. Autoradiograms were quantified with an image analyser: the density of the receptors was measured and the number of receptors per labelled structure was estimated. No differences were detected in the hypothalamus, the hippocampus, and the brainstem between both groups, while in the thalamus, AVP-R were much more numerous in obese than in lean rats: the number of AVP-R was 40% higher in the thalamic ventromedial nucleus, and 100% higher in the ventrolateral, the mediodorsal and the posterior thalamic nucleus. These four nuclei are essentially implicated in motor control. The physiopathological significance of these results is under investigation.


Our early evidence of recognition sites in the cerebral cortex for the neuropeptide vasopressin (Brinton et al. PNAS 1984, Chen et al. Hippocampus, 1993) suggested the existence of V1 vasopressin receptors. Based on these findings, we have pursued the signal transduction mechanism of these receptors in cultured cortical neurons using a selective V1 vasopressin receptor agonist. The dose-response of V1 agonist-induced accumulation of [3H]IP, was concentration dependent and showed a significant linear increase from 250nM (138%+16, p<0.01) to 1000nM (189%+16, p<0.01). A significant increase in the accumulation of [3H]IP, was observed within 25ms exposure to V1 agonist. Peptide specificity indicated that the closely related vasopressin metabolite peptides AVP47 and AVP45 also induced significant increases in [3H]IP, accumulation as did oxytocin. We further determined whether V1 receptor activation regulated the influx of calcium by conducting [45Ca] uptake analyses. Results of these investigations demonstrated that V1 agonist (250nM) induced a significant increase in [45Ca] uptake from the extracellular medium within 5 sec. V1 agonist-induced [45Ca] uptake dose-response analysis exhibited a nonlinear profile. These results demonstrate that V1 vasopressin receptor activation leads to calcium signaling in cortical neurons through the PI(2)-pathway and calcium uptake from the extracellular medium. Future studies will investigate V1 agonist-induced increases in intracellular calcium using calcium fluorescence analysis.

Supported by NIH grant 460365 to R.D.B.

T24.8 Vasopressin-Induced Specific Gene Expression in the Cultured Cortical Neurons. Qi Chen*, S. Schreiber and R.D. Brinton. Dept. Molecular Pharmacology & Toxicology, Univ. of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Ave., Los Angeles, CA 90033

Work from our laboratory has demonstrated the existence of V1a vasopressin receptors (V1aR) in cerebral cortex (Brinton et al, 1989, Yamazaki et al, 1995). In addition, we have found V1aR induces calcium signaling in cortical neurons and astrocytes (Son and Brinton, 1995). Downstream effects induced by V1aR activation are, however, still unknown. We have investigated specific gene expression induced by V1aR activation in cultured rat cortical neurons by using differential display PCR. Eight-day-old rat cortical neurons were cultured in serum-free media for 16 hours and treated with 250nM V1aR agonist for 15 min, 2 hours and 24 hours, respectively. Total RNAs were isolated and subjected to differential display PCR by using an anchor oligo(dT) primer and an arbitrary primer. Differential patterns were run on the polyacrylamide gels and visualized by silver staining or labelled by 32P-dATP. Our preliminary results indicate V1aR-induced specific gene expression. Further identification by Northern blot analysis and sequencing will be conducted. 

Supported by NIH grant MH66036 to R.D.B.

T24.9 V1a VASOPRESSIN RECEPTOR GENE EXPRESSION IN CULTURED CORTICAL NEURONS, ASTROCYTES AND OLIGODENDROCYTES. R.S.Yamazaki*, O.Chen, S. Schreiber, and R.D.Brinton, Dept of Molecular Pharmacology & Toxicology, 1 Dept of Neurology, 2 Univ. of Southern California, Pharmaceutical Science Center, 1985 Zonal Ave., Los Angeles, CA 90033

Our early evidence of recognition sites in the cerebral cortex for the neuropeptide vasopressin (Brinton et al. PNAS 1984, Chen et al. Hippocampus, 1993) suggested the existence of V1 vasopressin receptors. To date there has been no direct evidence of the existence of this type of cell in cerebral cortex of the V1 receptor. We pursued this question by using selectively enriched cultures of cortical neurons, astrocytes, and oligodendrocytes. Dissociated cortical cells derived from E16 rat brains were cultured in T150 plates in serum free medium. Neurons were collected after 4 days in vitro (DIV). Oligodendrocytes and astrocytes were collected separately after 21 DIV. Total RNA from each fraction was extracted and purified. To convert the RNA to cDNA and amplify the signal, RT-PCR was performed on each sample using 2 sets of 20mer oligonucleotides targeted to a vasopressin V1a receptor (V1aR) sequence ~350 bp and a vasopressin V1b receptor (V1bR) sequence ~500bp. Samples were electrophoresed and immobilized on nylon membranes. Hybridizations of the samples with labeled probes directed against V1aR and V1bR detected the presence of V1a cDNA, and the absence of V1bR cDNA, in all 3 cell types in the cerebral cortex. These data suggest that distinct vasopressin receptor types are localized in cortex and that within the cortex, the V1a receptor type is expressed in each of the 3 cell types. Vasopressin may have pleiotropic effects in the cerebral cortex. In situ hybridization studies to determine the magnitude of expression and proportion of each cell type that expresses V1aR are in progress.

Supported by NIH grant MH66036 to R.D.B.

T24.10 IDENTIFICATION OF OPIOID RECEPTOR-LIKE SEQUENCES AMONG DIFFERENT SPECIES. R.L. Fed, Keith Jr., C.U. Evans, Department of Psychiatry and Biobehavioral Sciences, University of California at Los Angeles School of Medicine, Los Angeles CA 90024-1759

The DNA sequences of members of the opioid receptor family (mu,delta kappa and the orphan ORL-1) have regions highly conserved - a conservation that is retained in human, rat and murine sequences. Areas that are highly homologous within this family of G-protein coupled receptors include the intracellular loops and the transmembrane domains. In order to gain insights into the evolution of the opioid receptor family, degenerate primers were designed according to sequences highly conserved within the family and polymerase chain reaction (PCR) was used to screen genomic DNA from a number of different species. DNA was assayed both from the proteolophid and ophiidophid branches of the metazoon phylogenetic tree. The specific products from PCR were subcloned into pCRII and sequenced. Opioid receptor-like sequences were obtained from beef, chicken, fish and frog. No specific opioid-like sequences were obtained from C-elegans or drosophila. In frog, we obtained three different receptor sequences that were close to these rat homologous but not identical to the mu, delta, and kappa opioid receptors previously isolated from mouse. Among the three receptors identified from frog, the kappa receptor was most divergent from the murine counterpart in the PCR sequence that was analysed. Lower species are presently being investigated to more fully understand the evolution of the opioid receptor family. Supported by NIDA DA05010 and W.M. Kneck Foundation.
724.11 A NOVEL BOMBEISIN-LIKE-PEPTIDE ISOLATED FROM THE FROG RANA CATERIEBANA. B.J. Barry, S.P. Negalla, M.S. Smith, E.R. Spindel. Div. Neuroscience, Molecular Medicine, Scripps Research Institute, La Jolla, CA 92037.

Bombesin was originally characterized in the skin of Bombina bombina. Many bombesin-like peptides (BLPs) have since been isolated from various amphibians and mammals. The BLPs are usually divided into three subfamilies: the GRP subfamily, the NMB subfamily, and the bombesin-related peptide (BRP) subfamily which contains bombesin, ranatensin, and phytoallotropin. These peptides have been distinguished from the bombesins by the presence of a Phe rather than a Leu as the penultimate C-terminal amino acid; however a Phe form of bombesin has recently been cloned. The discovery indicates that Bombesin and ranatensin may be more closely related than thought, while also increasing the apparent complexity of BLPs. Previously Ranatensin C, which is highly homologous to ranatensin, was characterized in Rana catesbeiana. Using HPLC and gel electrophoreses we have identified a new BLP, Ranatensin-V, in R. catesbeiana skin. Ranatensin-V has a valine in the penultimate position-the only BLP so far described to have this feature.

Ranatensin-1 NMR and HPLC, Electron spray mass spectrometry, Protein sequencing.

724.12 IDENTIFICATION OF NOVEL CHEMOKINE-LIKE RECEPTORS IN THE MAMMALIAN CNS BY PCR. M.E. Charlebon*, S.R. Marsh, A.M. Ciabarra and H.S. Dunsford. Department of Molecular Pharmacology, Yale University School of Medicine, New Haven, CT 06519.

Over the past several years our laboratory has been focused on the function of several brain regions implicated in the etiology and treatment of psychiatric disorders. Among these brain regions the molecular targets of actions of psychotropic drugs within these brain regions we have initiated studies to identify novel receptors belonging to the G-protein coupled receptor superfamily. Using degenerate primers corresponding to regions highly conserved among receptor subfamilies, our laboratory has identified several cDNA fragments encoding putative G-protein coupled receptor brain regions including the ventral tegmentum (VT) and locus coeruleus (LC). Sequencing analysis of the novel clones VTR 15 and LCR 12-12 has revealed a homology at the amino acid levels to the IL-8 and Monocyte Chemoattractant Protein (MCP) receptors, respectively. The distribution pattern and relative expression levels of the mRNAs encoding these clones was determined by RNase protection assay and northern blot analysis. The highest expression levels were detected in peripheral tissues (spleen, lung, heart, liver) and lower levels of mRNA expression were apparent in CNS regions (thalamus, pons, cortex, hippocampus, and cerebellum). We are currently isolating full length cDNA and characterizing the properties of these receptors. These studies demonstrate the existence of several novel chemokine-like receptors in brain and suggest a role for chemokines in the regulation of brain function.


Chemokines are a class of pro-inflammatory peptides that are important mediators of leukocyte migration. While these agents have been well characterized for their effects on peripheral systems and models of peripheral inflammation, little is known about the role or function of these chemokines in the central nervous system (CNS). Receptors for some of the chemokine peptides have been identified by molecular cloning and are members of the large gene superfamily of G-protein coupled receptors (GCRs). Here we report the identification of a novel rat chemokine receptors, amplified (by the polymerase chain reaction) DNA fragments encoding several novel members of the GCR superfamily. These fragments were used subsequently to screen rat genomic DNA libraries. Sequence analysis of one of these genomic clones (gCrc13c) revealed a translational open reading frame of 354 amino acids and a predicted extracellular domain 7% identical to the human monocyte chemotactic protein 1 (MCP-1) receptor, with the greatest divergence in the N-termius and second extracellular loop. Northern analysis of cultured rat microglia demonstrated the presence of mRNA hybridizing to Grc13c DNA. Treatment of the cultures with Interferon-γ resulted in a dramatic up-regulation of this mRNA. We are hypothesizing that the rat CNS (and specifically microglia) express functional MCP-1 receptors.


A recent report that a CCK peptide analog, Compound I, is an agonist prompted us to investigate the nature of its interaction with the CCK-A receptor. We compared the effect of Compound I on the binding of an agonist, [3H]CCK8, and an antagonist, [3H]MK329, in rat pancreas membranes. Scatchard analysis confirmed that CCK8 competitively blocked [3H]MK329 binding in this preparation, with 10 mM CCK8 increasing the dissociation constant from 0.3 ± 0.2 m of 2 ± 2 m with an effect on Bmax Compound I competitively displaced 0.5[CCK8 binding. In the presence of 5 mM Compound I, the apparent Kd for [3H]CCK8 shifted from 20 pM to 225 ± 25 m of 100 m Compound I was a non-competitive inhibitor of [3H]MK329 binding, decreasing Bmax from 244 pmol/gm tissue to 78 pmol/gm tissue. These data show that two components, both of which compete with CCK8 for the CCK-A receptor, are non-competitive with each other. The finding that Compound I binding is non-competitive with [3H]MK329 whereas CCK8 binding is competitive with [3H]MK329 indicates that the interaction of agonist with the CCK-A receptor is proneotropic. From our studies Compound I and MK329 occupy separate but allosterically linked domains of the CCKB binding region in the A receptor.

724.16 CHARACTERISTICS OF RADIOIODINATED BINDING BY SCIENZTILLATION PROXY ASSAY TO ANGIOTENSIN II TYPE 1, DOPAMINE D3, AND 5-HYDROXYTRYPTAMINE TYPE 1A RECEPTORS EXPRESSED IN COS-7 CELLS USING THE BACULOVIRUS SYSTEM. G. O'Brien*, A.J. Harvey, M. Donald*, A. Patel, and N.D. Cook. 1. American International plc, Cardiff Labs., Whitchurch, Cardiff, Wales, CF4 7YJ. 2. Biologial Inc. 174 rue William- Montebé, (Quebec), Canada H3J 1R3

Scintillation Proximity Assay (SPA) technology was used to characterise the binding of radioligands to various human recombinant receptors expressed in SF 9 insect cells via the baculovirus system. It was possible to couple the SF 9 insect cells containing the baculovirus-expressed recombinant human angiotensin agonist (WGA) and polyethylene coated SPA beads. The SPA beads contain scintillant which detects low energy emissions (i.e. beta-particles from 32P or Angiotensin from 125I) only when the radioreceptor is in close proximity to the bead. In accord with receptor binding assays it is not necessary to separate receptor-bound ligand from free, so only radiolabelled to bound to receptors coupled to the bead causes light to be emitted. In these experiments the binding characteristics were determined by SPA using WGA SPA beads and compared to those determined using traditional filtration methods. Three different receptor/ligand systems were examined in detail. [3H]Barclagenolin II binding to angiotensin II type 1 receptors, [3H]epidepride binding to dopamine D2 receptors and [8-3H]dihydroergotamine type 1A receptors to 5-hydroxytryptamine type 1A receptors. Comparable Kd values were found when saturation binding experiments were performed by both SPA and filtration methodologies. These studies demonstrate that it is possible to use SPA to determine recombinant receptors expressed using the baculovirus insect cell system.
724.17

Concerted releasing factor (CRF) is an early signal in stress response. A functional CRF receptor which increases adenylyl cyclase activity on stimulation by CRF has been found in the hypothalamus and the pituitary gland. CRF receptor cloning has been developed which show similar binding affinities and biopotencies when compared to hCRF(1-41) and the antagonist [D-Phe 12, Nle(2,38)]hCRF(12-41), respectively.

In a PCR based approach we could demonstrate that CRF1 receptor is expressed in Y79 cells. Furthermore, three different variants of this receptor type were found. The first variant encodes the previously cloned CRF1a receptor polypeptide. The two additional variants are novel. Both have deletions of different lengths in the extracellular N-terminal domain. The presence of different receptors is consistent with our binding data performed on Y79 membrane homogenates, that favor the existence of high- and low-affinity binding sites.

For the identification of the signal binding site with a potent photocytovactivatable CRF analogue, HEK 293 cells were transfected with the rat CRF1 receptor. The CRF binding constant did not deviate significantly from the corresponding values of pituitary or Y79 cell receptors. However, the CRF receptor density was significantly increased in HEK cells when compared to the receptor density in Y79 cells. The ongoing production of stable cell lines expressing functional CRF receptors at higher level will facilitate the structure-activity relationship (SAR) studies with CRF agonists and antagonists.

724.18
IRRRESSIBLE ANTAGONISM OF THE GONADOTROPIN-RELEASING HORMONE RECEPTOR TO EVALUATE "SPARE RECEPTORS" IN TRANSIENT TRANSFERENCE SYSTEMS. D. Kusmartsev, J.C. Stetten, Fishberg Center for Neurobiology and Dept. of Neurology, Mount Sinai Medical Center, New York, NY 10029.

Transient transfection systems are frequently employed to study the coupling of wild type (WT) and mutant receptors. However, EC50 values obtained in functional studies depend on the level of receptor expression per cell. An approach sometimes used to determine the relationship between EC50 and receptor expression following transient transfection is to alter the amount of receptor DNA being transfected into cells. However, the interpretation of results obtained is not conclusive because it is not known whether increasing the amount of transfected plasmid causes a change in the expression of receptors per cell or in the number of cells expressing the receptor. We have evaluated the relationship between measured EC50 and the concentration of the gonadotropin-releasing hormone receptors (GnRH) in COS-1 cells by parallel irreversible antagonism. Functional receptors were progressively eliminated by pretreating cells with 2,4,6-trinitrobenzenesulfonic acid (TNBS). With the greatest reduction in receptor concentration, EC50 values obtained from control and GnRH-IR50 was observed. (Supported by NIH RO1 DK-46943 and G3-5615 training grant)

724.19
NOVEL ACTIVATION AND INTERACTION OF SYMPATHETIC NEURON SIGNAL TRANSDUCTION PATHWAYS BY PITUITARY ADENYLATE CYCLASE POLYPEPTIDE (PACAP). K. Braat, and V. M. M. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405. Pituitary adenylate cyclase activating polypeptides (PACAP-27 and -38) belong to the VIP/Pacifichonocytate family of bioactive peptides and have diverse neuroendocrine regulatory effects. The PACAP-selective type I receptor is positively coupled to adenylyl cyclase and phospholipase C (PLC); splice variants of the type I PACAP receptor were differentially stimulate adenylyl cyclase and PLC. In the current studies, cultured superior cervical ganglion (SCG) neurons, which expressed predominantly the HOPI cassette-containing type I PACAP receptor isoform, displayed potent and efficacious stimulation of catecholamine and NPY secretion by PACAP peptides. Unlike previously studied tissues, SCG inositol phosphate and cyclic AMP production were potentiated by both PACAP-27 and PACAP-38. PACAP-38 was more potent than PACAP-27 in stimulating SCG cyclic AMP levels; PACAP38 exhibited a half-maximal effect at 0.4 nM compared to 4.0 nM for PACAP 27. PACAP 27, however, was more efficacious than PACAP 38 in stimulating inositol phosphate production exhibiting a half-maximal response at approximately 0.5 nM. PACAP-27 or PACAP-38 elicited interactions of these two signaling pathways were investigated using the PLC inhibitor U73122 and/or the adenylyl cyclase inhibitor SQ22536. U73122 attenuated both PACAP-27 and PACAP-38-stimulated inositol phosphate production, while SQ22536 had no effect. In contrast, PLC inhibition potentiated PACAP-27 and PACAP-38-stimulated cyclic AMP production. These results indicate that the concerted interactions of these two signal transduction pathways contribute to SCG responses to PACAP. (Supported by HD67448 and NS21636 (VM) and AHA4015440 (KMB)).

724.20

Neuroblastoma-derived cell lines express different neurotrophin receptors and represent an useful model to investigate their molecular functions in differentiated cells which acquire several phenotypes of mature neuronal cells. We have examined a series of human neuroblastoma cell lines with the aim to detect, by polymerase chain reaction, any mRNA coding for the calcitonin receptor by using two primers complementary to an unique sequence of a cDNA described by Kusevitz et al. (Mol. Pharmacol. 46,246.1994). Unique analysis of reverse-transcribed total RNA, provided evidence that mRNA coding for this receptor occurs in IMR 32 cells. In these cells, differentiated with 1 nM dibutyl cAMP for 12-14 days and loaded with fura-2, human calcitonin (IC50=10-8M) and salmon calcitonin (IC50=10-9M) antagonized Ca2+ entry evoked by high extracellular K+ (50 mM) or ATP (1 mM). This response was not observed following removal of extracellular Ca2+ or in the presence of α-conotoxin GVIA (20 nM). Therefore, differentiated IMR 32 cells represent an useful tool to study the gene expression and molecular interactions of human calcitonin receptor in neuronal cells.

NEUROTRANSMITTER PROCESSING

725.1
APLYSIA CARBOXYPEPTIDASE E: MOLECULAR CLONING, STRUCTURE, AND CELLULAR LOCALIZATION. G. T. Nagle. W.R.A. van Heumen, R. Rodriguez and X. Pan. Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555 and Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane, Australia.

The abdominal ganglion neuroendocrine bag cells of Aplysia express the egg-laying hormone (ELH) gene and process the resulting polypeptide ELH precursor at multiple sites. Carboxypeptidase E (CPE)-like removal of C-terminal basic amino acids has been previously detected in neural tissue of Aplysia. To characterize this process in greater detail, an abdominal ganglion cDNA library was constructed and screened using a CPE-related PCR product generated from the bag cells. The longest clone that was isolated contained the entire open reading frame and encoded a preproenzyme that was most closely related to vertebrate CPE. The sequence of Aplysia CPE is currently being examined by in situ hybridization. The C-terminal region of vertebrate CPE contains the membrane anchor and may contribute to the sorting of this protein into the regulated pathway. Interestingly, the C-terminal region of Aplysia CPE showed a low sequence identity with the C-terminal region of vertebrate CPEs.

725.2

The abdominal ganglion neuroendocrine bag cells of Aplysia express the egg-laying hormone (ELH) gene and process the ELH precursor to generate amidated ELH and other peptides. In addition, these cells and other abdominal ganglion neurons express a homolog of neuropeptide Y that is also amidated. To characterize Aplysia peptidylglcine α-amidating monooxygenase (PAM), an abdominal ganglion cDNA library was constructed and screened using a PAM-related PCR product generated from the bag cells. The clone that was isolated encoded a PAM-related enzyme that was most closely related to vertebrate PAM. These sequences. The cellular localization of Aplysia PAM is currently being examined by in situ hybridization.

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725.3
SUBLITIN LIKE ENZYMES PC2, PC1/PC1 and FURIN IN UNDIFFERENTIATED AND DIFFERENTIATED HL-60 CELLS. Ondale Vindrola*, Maria Claudia Kicz and Maria Rosa Padron. *Instituto de Fisiologia Celular, Universidad Autonoma de Puebla, Puebla Mexico. 1 Instituto de Investigaciones Medicas. Facultad de Medicina. Universidad de Buenos Aires. Buenos Aires, Argentina.

Prohormone converting enzymes PC2 and PC1/PC1 (PC1) have been detected in rat neutrophils and alveolar macrophages, respectively. In order to ascertain in which stages of the differentiation process the enzymes are produced, we studied PC2, PC1 and furin content in undifferentiated (UD) and differentiated (D) human promyelocytic leukaemic cell line HL-60. PC2, PC1 and furin were assayed by gel electrophoresis followed by in situ zymography in 15% SDS-PAGE gels of HL-60 cell lysates. The expression of the neuropeptide-like (NL) and macrophage-like (ML) D-HL-60 cells. 68 kDa PC2 protein was detected in UD-HL-60 cells and its levels decreased dramatically in NL and ML-D-HL-60 cells. Comparative levels of expression were observed in NL-D-HL-60 cells and peripheral human neutrophils. Furthermore, a decrease of PC2 content occurred in peripheral rat neutrophils when compared with enzyme levels in rat bone marrow neutrophils. High concentrations of 66 kDa PC1 protein were detected in UD- and D-HL-60 cells, reaching the highest values in ML-D-HL-60 cells. PC1 did not appear in human peripheral neutrophils. 97 kDa furin was not found in HL-60 cells nor in human peripheral neutrophils. However, furin antibody recognized the 66 kDa PC1 processing, reproducing the results obtained with PC1 antibody. HL-60 is a regulated non-codocrine and non-nervous cell, and contained high levels of PC1 and PC1 enzymes, with the absence of furin. These results suggest that PC1 enzyme may be involved in the processing of proproteins in early stages of HL-60 differentiation processes, while PC2 may participate in early as well as in late stages of HL-60 differentiation, especially in ML cells.

725.5

The predominant form of cholecystokinin (CCK) in the brain is CCK 8, the second most abundant neuropeptide next to NPY. CCK 8 is an amino acid peptide produced from a larger pro CCK precursor and has been implicated in a number of physiological effects including satiety, anxiety, memory and analysis. A number of posttranslational modifying enzymes are involved in the biosynthesis of bioactive CCK 8 including intraneuronal sulfatransferases, endopeptidases and amidating enzymes; the identification of many of these enzymes are still under investigation. Candidate candidates for CCK 8 and PC2, stabilizing enzymes of the furin family that cleave peptides at monobasic or dibasic residues. Evidence suggesting roles for PC1 and PC2 in pro CCK processing include similar tissue distribution patterns and effects on both the expression and the processing of both PC1 and PC2 in several tissue culture cell lines that have the ability to process pro CCK to CCK 8.

In order to further evaluate the role of PC1 and PC2 in CCK processing, we have inhibited PC1 or PC2 by constitutively expressing antisense message in the murine intestinal tumor cell line STC-1, which produces not only CCK 8 but also CCK 21, the predominant form of CCK found in rat intestine. We have identified lides that show expression of antisense message by Northern hybridization and significantly reduced levels of PC1 or PC2 proteins levels by Western analysis. By radiomunnoassay of G-50 chromatography fractions, preliminary data suggests that the inhibition of PC1 in STC-1 cells specifically inhibits the production of CCK 8 and inhibition of PC2 in STC-1 cells specifically inhibits the production of CCK 22.

These data suggest the possible role of both PC1 and PC2 endopeptidases in the processing of pro CCK. Supported by NIH grant NS 31602.

725.7
MOLLUSCAN PROHORMONE CONVERTASES: STRUCTURAL DIVERSITY IN THE CENTRAL NERVOUS SYSTEM OF LYMNAEA STAGNALIS. J.B. Smillie*, S. Spitzer, J. Klumpman and W.P.M. Gerritsen. Graduate School of Neurosciences Amsterdam, Institute of Neurosciences, Faculty of Biology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands.

In the central nervous system (CNS) of the mollusc Lymnaea stagnalis various prohormones have been characterized, from which (sets of) different neuropeptides can be generated by proteolytic cleavage and posttranslational modifications.

In order to address the enzymes involved in the processing of neuropeptide prohormones we have used a PC1/PC2 antisense approach and characterized cDNAs corresponding to three different putative endopeptidases. One cDNA encodes a protein of 655 residues with an overall sequence identity of ~60% with the catalytically active vertebrate PC2, and appears to be the Lymnaea PC2 (LPC2). Expression of the LPC2 gene was exclusively found in neurons of the CNS, and the northern blot analysis showed 3.0 and 4.8 kb transcripts. Two other cDNAs were cloned using PCR and furin specific primers and were tentatively called Lfur1 (968 amino acids) and Lfur3 (837 amino acids). Lfur1 shows highest sequence identity to human furin (72%) in the catalytic domain and also in the C-terminal region is highly conserved. Lfur2 is structurally related to furin and shows ~70% homology with the catalytic domain whereas Lfur3 and PC2, whereas the sequence conservation in the C-terminal part of the protein is low. The Lfur1 gene is expressed in a broad range of tissues, and several sizes of transcripts can be detected. Expression of the Lfur2 gene is also found in this tissue and shows a single transcript of ~6.0 kb. Immunochemistry has indicated that the various Lymnaea convertases show cell-specific expression, that can be related to the maturation of specific prohormone substrates.

725.8

Regulation of prohormone convertase mRNA expression was studied in the neureoptype producing cell line WE 4-2, a rat medullary thyroid carcinoma line, to examine the effect of elevation of cyclic AMP (cAMP) second messenger pathway on prohormone convertase 1 (PC1), PC2 and furin mRNA levels. WE 4-2 cells were treated with the phosphodiesterase inhibitor IBMX (0.5-10mM). Treatment with 2-methylthio-3-thyrate 13-acetate (PMA, 0.5 µM) was used to detect if the protein kinase C (PKC) pathway regulates prohormone convertase mRNA levels. Messenger RNA (mRNA) levels were quantitated using Northern analysis in combination with cDNA hybridization probes, rat (I) PC1, PC2 and furin. In the cell line activation of the cAMP pathway increased mRNA levels of all three prohormone convertases. PC1 mRNA levels were increased 1.4-fold after 3 hour treatments and 1.5-fold after 6 hour treatments. PC2 mRNA levels increased 1.6-fold and 1.8-fold and furin mRNA levels increased 1.5-fold and 1.8-fold, after 3 and 6 hours respective. Treatment with PMA or PMA combined with IBMX did not produce significant changes in PC1, PC2 or furin mRNA levels. This pattern of regulation differs from one seen previously in the human medullary carcinoma cell line N-MC1/IXC (Soc. Neurosci. Abs. 19, 1993), suggesting that prohormone convertases may be differentially regulated by cAMP and PKC mechanisms in a tissue specific manner. Supported by N.I.H. # MH 46200.
725.11 EXPRESSION OF PROENKEPHALIN (PE) AND IN VITRO PROCESSING OF PURIFIED PE BY THE PROHORMONE THIOL PROTEASE (PTP) FROM THE RAT Chromaffin Cell Line AtT/20, J.P. Hoek, M.R. Mueller, M.D. Muralidharan, Dept. of Biochemistry, Uniformed Services Univ., Bethesda, MD, and Dr. Biochemistry, Med. College of Wisconsin, WI.

The prohormone to thiol protease (PTP) is a novel cysteine protease involved in proenkephalin processing. This study examined PTP processing of proenkephalin (PE) to intermediate products, and assessed kinetics of PE processing. Recombinant PE was obtained by high expression in E. coli. PTP was then purified by DEAE-Sepharose, gel electrophoresis, and reverse-phase HPLC. Authentic purified PE was confirmed by amino acid composition analyses and peptide microsequencing. In time course studies, PTP converted PE (12 μM) to intermediates of 22.5, 21.7, 12.5, and 11.0 kDa that represented NH2-terminal fragments of PE, as assessed by peptide microsequencing. Differences in Mr's of the 22.5, 21.7, 12.5, and 11.0 kDa products reflect PTP processing of PE within the COOH-terminal regions of PE, which resembles the N-terminal structures of PTP substrates of 12.5, 11.0, and 8.5 kDa resulted from PTP cleavage between Lys-Arg at the COOH-terminus of (Met)enkephalin-Arg6-Gly8-Leu9-PE. PTP has a Km(app) value of 18.6 μM PE and Vmax(app) of 1.98 mmol/min/mg. These kinetic constants are consistent with intragranular levels of PE and PE-products. Results demonstrating PTP conversion of PE to intermediates resembling those in vivo, and kinetics that are compatible with in vivo PE processing, implicate a role for PTP in PE processing.

725.12 INITIAL PROCESSING OF PROENKEPHALIN IN CHROMAFFIN CELLS OCCURS AT FOUR CENTRAL AND PERIPHERAL ENZYME ACTIVITIES. S.P. Wilson, F. Liu, and P.R. Houser, Dept. of Pharmacology, University of South Carolina School of Medicine, Columbia, SC 29208.

Processing of proenkephalin was examined in bovine chromaffin cells using recombinant plasmin containing the human proenkephalin (HhPPE) cDNA under the control of the human plasminogen promoter. The timing of PE production was examined using antiidiotype antibodies specific for PE. Results indicated that the proenkephalin cDNA was expressed in chromaffin cells, and that PE production was initiated within 2 h. Immunoreactive PE was detected by 2 h, and PTP activity was detected by 4 h. These results indicate that PTP activity is necessary for the initial processing of proenkephalin.

The neuropeptide substance P (SP) interacts with nigrostriatal and mesocorticolimbic neurons to modulate dopamine (DA) release. Dopaminergic drugs have been shown to decrease the level and mRNA of SP following subchronic administration. Additionally, our laboratory has shown that the metabolism of SP is altered in a regionally specific manner following 7 day administration of typical antipsychotics haloperidol (1mg/kg) and chlorpromazine (20mg/kg) suggesting altered neuropeptide activity (J. Pharmacol. Exp. Ther., in press). In the present study, the effect of subchronic (7 day, i.p.) administration of typical antipsychotics and selective dopaminergic receptor agonists and antagonists on SP degradation was determined using intact rat frontal cortex (FC), caudate-putamen (CP) and nucleus accumbens (NA) slices.

In contrast to the decrease in SP degradation in CP and NA following typical neuroleptics, the nonselective DA antagonist apomorphine (5mg/kg, bid) significantly increased SP metabolism in NA while the typical antipsychotics clonidine (20mg/kg) had no effect on SP metabolism in any region studied. Selective DA receptor compounds were used to determine through which receptor subtype haloperidol, chlorpromazine and apomorphine produce their effect on SP metabolism. Neither the selective DA1 antagonist SKF 38393 (1 or 5mg/kg, bid) or the DA2 antagonist SCH 23390 (0.5 or 2.3 mg/kg, bid) had an effect on SP degradation. Interestingly, the DA2 antagonist sulpiride (100 mg/kg, bid) was also without significant effect. Therefore, these data suggest that neuropeptide-induced alterations of SP-metabolizing neuropeptides are mediated through additive interactions at multiple DA receptor subtypes. (Supported by NIMH grant MH42600 and an AFPE fellowship.)

OPIOIDS: ANATOMY, PHYSIOLOGY AND BEHAVIOR III

726.1 LOCALLY ADMINISTERED MORPHINE REDUCES VENTRAL PALLIAL OPIOID PEPTIDE RELEASE. M.F. Olve* and N.T. Meldrum.

Neuroscience Ph.D. Program & Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024, USA.

Previous work from our laboratory has demonstrated a morphine-induced elevation in pallidal extracellular opioid peptide levels following peripheral administration (NeuroReport, in press). In an initial attempt to determine the site of action for this effect, morphine was locally administered into the ventral pallidum and changes in opioid peptide release in this region were studied in freely moving rats using in vivo microdialysis coupled to highly sensitive radioimmunoassay procedures. We have previously shown that the opioid peptides recovered from pallidal dialysates are primarily met- and leu-enkephalin (Neuroscience 33:549-557, 1989). Male Sprague-Dawley rats were implanted with guide cannulae and allowed at least 4 days of recovery prior to in vivo microdialysis. Pairs of dialysis probes (250 mm in length) were implanted into the ventral pallidum. Following an overnight equilibration period, dialysis samples were collected at 20 min intervals. When morphine was incorporated into the perfusion medium for 30 min at 2 nM intervals, opioid peptide release was slightly but not significantly decreased with respect to baseline during each 90 min post-injection period at 10 µM (16.7±18.5%, n=7) and 100 µM (5.5%, n=6) concentrations. However, when 100 µM morphine was incorporated into the perfusion medium for 120 min at 4 h intervals, opioid peptide release was significantly reduced during each 2 h period post-injection, with the second administration producing a more profound (46±11.9%, n=6) decrease. These data demonstrate that opioid peptide release in the ventral pallidum is suppressed by local administration of morphine and suggest that this peptide release in perhaps under control of presynaptic µ opioid receptor autoregulatory mechanisms. Supported by DA50101, DA50334 & the W.M. Keck Fdn.

726.3 POTASSIUM AND 4-AMINOPYRIDINE EVOKE RELEASE OF STRIATAL DOPAMINE AND HIPPOCAMPAL NOREPINEPHRINE DURING MORPHINE WITHDRAWAL. Kenneth Graint*, Stefan D. Schimmer and John Woodward Program in Clinical Pharmacology, Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903.

Microdialysis studies have shown that opiate withdrawal can diminish in-vivo levels of extracellular dopamine (DA). Using a striatal slice preparation, previous studies have demonstrated that the electrically evoked release of DA is decreased for periods of up to 7 days of withdrawal. We attempted to extend these findings by examining the potassium (K') and 4-aminopyridine (AP) stimulated release of DA in the stratum and norepinephrine (NE) release in the hippocampus following withdrawal from morphine dependence.

Wistar rats received continuous infusions of morphine sulfate (MS) at 6 mg/kg/hr delivered by subcutaneous osmotic pumps (no. 6) or sham operations (no. 6) for 7 days. Following 2 or 15 days of withdrawal, animals were sacrificed by decapitation and the striatum and hippocampus were removed and cross cut to 350 µm slices with a McIlwain tissue chopper. Tissue slices were washed extensively and then loaded with [3H]DA and [3H]AP. After determining baseline levels of [3H]DA release, slices were exposed to 25 mM K' or 300 µM AP (stimulation 1:1), with a second stimulation applied after an additional baseline period (32). Tissue from morphine (MS) treated and control (CTL) subjects demonstrated similar levels of neurotransmitter release. Mean and standard deviation for striatal tissue 51 was 9.5±2 ± 3 and 10.4 ± 2.1 for CTL and MS K' stimulated with 6.0% ± 2.1 and 7.8% ± 3.0 for CTL and MS AP stimulated with 7.5% ± 2.3 and 7.0% ± 0.5 for CTL and MS K' stimulated with 3.9% ± 1.0 and 4.7% ± 1.7 for CTL and MS AP stimulated. In conclusion, neither K' or AP evoked release of striatal DA or hippocampal NE were altered during MS withdrawal.

726.4 EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON ACUTE MORPHINE AND CLONIDINE WITHDRAWAL RESPONSE IN THE RAT LOCUS COERULEUS. S. Hall, S. Dugan, B. Milne, K. Jhamandas*, Departments of Pharmacology & Toxicology, and Anesthesia, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

Activation of NMDA and non-NMDA receptors contributes to the hyperactivity of noradrenergic neurons of the locus coeruleus (LC) associated with opioid and non-opioid drug withdrawal syndromes. Using an in vivo voltagemeter approach, we have examined the role of nitric oxide (NO), which mediates NMDA receptor function, in this hyperactivity. In the anesthetized rat, an acute intraventricular injection of morphine (10 µg) or clonidine (10 µg) suppressed catecholamine (DA, NE) and N-acetyltyrosine (NA) turnover in the LC of rats. This effect was reversed by the nitric oxide synthase (NOS) inhibitor, L-NAME. We have examined the LC withdrawal response. This treatment, however, did not influence the inhibitory effect of morphine or clonidine on activity. The results suggest that NO plays a role in the genesis of acute morphine and clonidine withdrawal at the level of LC. (Supported by Medical Research Council of Canada.)
OPIOIDS: ANATOMY, PHYSIOLOGY AND BEHAVIOR III

726.5
EFFECTS OF THE NO SYNTHASE INHIBITOR, L-NAME ON MORPHINE TOLERANCE. AN EEG STUDY IN THE RAT
X Guo, L. R. King, A. Matta and J. E. Forsythe. Pharmacology and Toxicology, Univ. Maryland Sch. Pharmacy, Baltimore, MD 21201.

A previous study has shown that N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, attenuated the development of tolerance to the analgesic effects of morphine in mice (Majed et al., 1993). In this study, electroencephalography (EEG) was used to investigate the effect of L-NAME on morphine tolerance in three groups of female Sprague-Dawley rats bearing chronic i.v. cannulae and cerebroelectrodes. Groups I, II and III received chronic saline, chronic morphine, or chronic morphine plus L-NAME (5 mg/kg/day), respectively. Cumulative dose-response curves for EEG total power and edge frequency were generated in each group by giving morphine (2.5, 5, 10, 20, 40 and 80 mg/kg, i.v.). Relative to Group I, Group II demonstrated morphine tolerance as shown by decreased EEG total power and increased edge frequency. Co-administration of L-NAME (Group III) did not prevent morphine tolerance except at the highest morphine dose. Similar to the report of Rauhala et al. (1994) for hyperthermic & hormonal responses in the rat, our data likewise suggest that tolerance to the EEG effects of morphine are not attenuated by L-NAME.

726.7

We have previously shown chronic co-treatment with MK-801 does not prevent the development of morphine tolerance in the isolated spinal cord of the neonatal rat. In the present study we tested the effect of acute MK-801 on the expression of tolerance in this preparation. Acute morphine reduces the area of the ventral root potential (VRP) without reducing the peak amplitude. MK-801 (300nm) alone has no effect on the peak amplitude or area of the VRP, but enhances the morphine induced depression in saline treated neonates. Thompson et al., (1992) have characterized various components of the electroevoked VRP (VRP) relating to various types ofafferent input. In the present study we observed the peak of the prolonged component of the VRP which occurs 2 seconds after a single shock stimulation of the dorsal root of sufficient intensity to activate C-fibre afferents. MK-801 (300nm) alone had no effect on the area of the VRP by reducing the prolonged late portion of the response. Acute MK-801 with morphine in the above concentrations, decreased the 2 second peak by 23, 37, 55 and 69 percent respectively in saline treated neonates. In chronic morphine treated neonates acute MK-801 (300nm) with morphine in the above concentrations decreased the 2 second peak by 11, 34, 53, 64 percent respectively. These results show, in the presence of morphine tolerance, acute MK-801 still acts in synergy with morphine to reduce the peak of the prolonged VRP. This tolerance to this synergistic effect is not produced by chronic morphine administration.

726.9
EFFECT OF NALOXONE AND NEUROPEPTIDE F F (NFPP) ON THE EXPRESSION OF C-POS IN THE BRAIN OF MORPHINE-DEPENDENT RATS. K.H. Jamhari, K.H. Harris, M. Subtel and J.H. Jamhari. Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario & Department of Medicine (Neurology) & Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada.

NFPP has been designated as an endogenous morphine modulatory peptide with a potential to exert anti-opioid or opioid-like effects. To assess the potential of this peptide as an anti-opioid, we examined the effect of NFPP, 1DME (a synthetic NFPP analog resistant to degradation), and naloxone on behaviour and CNS expression of Fos in morphine-dependent and control rats. Animals received intracerebroventricular infusions of morphine (15ug/h) or saline for 5 days, following which central injections of either naloxone, NFPP, or 1DME were made. In saline-infused animals naloxone prevented the behavioural and morphine induced expression of Fos in the brain. Inoculation of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injections of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injection of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injection of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injection of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injection of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. In NFPP and 1DME groups, central injection of Fos-positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of naloxone (0.5ug) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity.

726.10
CHRONIC EXPOSURE TO MORPHINE ATTENUATES THE EXPRESSION OF INTERLEUKIN-1\(b\) CONVERTING ENZYME IN THE RAT BRAIN. S.L. Chim, D. B. Chang, G. Wu, J. Giff, J.E. Katz and N.A. Patel. Department of Physiology, Seton Hall University, South Orange, NJ 07079, VA Medical Center and Tulane Medical School, New Orleans, LA.

Alterations in the production, activity, or metabolism of interleukin-1 (IL-1), an immunocytokine, by exogenous factors may have modulatory effects on the neuroendocrine-immune system. We recently reported that chronic morphine treatment attenuates the expression of IL-1 converting enzyme (IL-1\(b\)-converting enzyme or ICE) in the rat hypothalamic paraventricular nucleus (Chang, et al 1994). In this study, we examined the expression of ICE in the rat hippocampus (Paxinos & Watson, 1986) using a polyclonal antibody that specifically recognizes the IL-1 converting enzyme (RT-PCR). Our data strongly suggest that the expression of ICE in both the hippocampus and hypothalamus of the rat is reduced by chronic morphine treatment. These data suggest that a decrease in ICE may mediate, or contribute to the attenuation of IL-1\(b\) expression in the rat brain following chronic treatment with morphine.

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726.11
CHRONIC MORPHINE (MOR) TREATMENT LEADS TO COMPARABLE REDUCTIONS IN RESPONSES OF GUINEA PIG STS NEURONS TO MOR, MORPHINE (MUS) AND 2-CHLORO-D-DEOXYGALACTOSE (2-CADO). C.J. Malanga, W.W. Fleming and D.A. Taylor*. Dept. of Pharmacology, WVU School of Medicine, Morgantown, WV 26506-9223.

Chronic implantation with MOR pellet led to a 5.6-fold submissivity of guinea pig nTS neurons to MOR and MUS and a 3.6-fold submissivity to elevation in extracellular K+. Since cumulative addition of either MOR or 2-CADO leads to acute desensitization in control preparations, the specificity of the chronic submissivity of opioid tolerant nTS neurons was examined using single units of the agonists. Acute, extracellular action potentials of individual nTS neurons were recorded and drug responses quantified as the percent reduction in frequency. Each neuron was exposed to MOR (1μM) and either MUS (0.5μM) or 2-CADO (1μM) with 15 minute washout between the two treatments to examine the magnitude of reduction in the inhibitory activity of each agent that was similar among brain slice preparations for each agonist employed. MUS produced 50% while MOR produced 75% inhibition of neuronal activity in placebo treated preparations. Chronic treatment with MOR reduced the inhibitory activity of MOR by 41% and that of MUS by 47%. 2-CADO reduced neuronal activity by 65% while MOR inhibited the activity of the same neurons by 60%. Chronic in vivo treatment with morphine reduced the inhibitory effects of these nTS neurons as using by 66% for MOR and 65% for 2-CADO. Thus, the development of tolerance to opioids in the guinea-pig brainstem is associated with a nonspecific change in responsiveness to a variety of agonists. The data are consistent with a cellular mechanism of adaptation involving general neuronal responsiveness, such as membrane properties, rather than specific receptors. Supported in part by NIH grant DA03773.

726.13

Previous research shows that intake of palatable foods alters morphine-induced analgesia. Some of these studies found an enhanced MIA after sucrose feeding whereas others showed a decreased MIA. The present study was designed to examine whether acute versus chronic exposure to sucrose solutions differentially alters MIA, and whether availability of water in addition to sucrose is necessary to enhance MIA. Experiment 1: Adult male Long-Evans VAF rats. All rats had ad lib access to ground chow and water. Ten rats were given a 32% sucrose solution in addition to water. The acute test phase took place after 5 hr of access to either sucrose or water alone. The chronic test phase took place after rats had been consuming sucrose for 3 wks. MIA was assessed with the tail-flick method. Injections of 2.5 mg/kg (p) of morphine sulfate were given in a cumulative dose paradigm every 5 min, until a final dose of 15 mg/kg was achieved. In the acute feeding test, sucrose suppressed MIA, whereas chronic intake of sucrose significantly enhanced MIA.

The second study examined if a choice between water and sucrose was necessary to alter MIA. Three diet conditions were used with 10 rats in each: water alone, sucrose alone, and water and sucrose. The rats were also fed ground chow. There was an acute test after 5 hr of fluid availability, and a chronic test after 3 wks. In the acute phase, sucrose alone reduced MIA relative to water and sucrose and water. During the chronic stage, however, exposure to sucrose regardless of a water choice enhanced MIA.

These results indicate that duration of exposure to palatable foods influences MIA. Acute exposure may release endogenous opioid peptides and induce tolerance to morphine, while chronic intake may alter opioid receptors.

726.15
HOMOLUMO vs. HETEROLUMO DESENSITIZATION OF RECEPTOR-MEDIATED POTASSIUM CURRENTS IN THE RAT LOCUS COERULEUS. S.L. Langare, C.D. Fisler and L.T. Williams, Volcani Institute, Oregon Health Sciences University, Portland, OR 97201.

μ-opioid and δ2-adrenergic receptors both activate the same potassium channels in rat locus coeruleus (LC). Acute or chronic treatment with opioid agonists induces desensitization of specific potassium channels with little change in the μ2 mediated response. We have performed intracellular recordings to make a quantitative comparison of desensitization to opioid and δ2 agonists in a brain slice preparation of rat LC. Drug-induced outward potassium currents were measured in neurons voltage-clamped at -60 mV. A supramaximal concentration of the opioid agonist met-enkephalin induced a profound homologous desensitization, but little heterologous desensitization to δ agonists or somatostatin. A supramaximal concentration of the δ3 selective agonist URI14304 showed relatively little desensitization. Current-voltage plots revealed that both the opioid and δ2 desensitization observed at -60 mV was due only to a reduction in potassium current. Opioid and δ2 desensitization could also be distinguished by muscarinic modulation. In the presence of muscarinic agonists, the rate and magnitude of opioid desensitization was increased, whereas δ2 desensitization was not altered. The acute desensitization examined here shares some characteristics with the desensitization observed in chronically morphine treated animals and may play a role in the initiation of chronic tolerance to opioids.

726.16
INTERACTION BETWEEN OPIOIDS AND EXCITATORY NEUROTRANSMITTERS IN THE MESENCEPHALON. J.L. Garcia*, S. Jolla, J.L. Garcia and J.L. Garcia, Dept. of Neurophysiology, The Scripps Research Institute, La Jolla, CA 92037.

Mu opioids can mediate reinforcing effects of heroin in the rat. This mediation may involve alteration of endogenous neurotransmitter systems. Medial prefrontal cortex (mPFC) is a significant component of reinforcer pathways, and the activity of its neuronal population has been previously shown to be modulated by both chemical and electrophysiologically administered opioids. In this study we sought to explore possible mechanisms for this control.

Extracellular recordings were made from neurons in the mPFC in halothane anesthetized rats. All the neuronal populations were anterior cingulate, prelimbic and infralimbic components of the mPFC. Systemic morphine (2.5 mg/kg, sc) and electrophysiologically-applied DAMGO, a μ opioid agonist (1 nM) were used to examine their effect on mPFC neuronal firing in response to electrophysiologically-applied acetylcholine (ACh, GLu, 100 nM). As expected, prior to the application of opioids, many mPFC cells were found to increase firing rates in response to both ACh and GLu. Systemic morphine attenuated or blocked the GLu-induced excitation in the majority of these cells, whereas the excitatory effect remained unaffected. However, some neurons exhibited a significant increase in response to ACh or GLu following systemic morphine. As noted previously, electrophysiological application of DAMGO decreased the spontaneous firing of most mPFC neurons. When applied concurrently with either ACh or GLu, this μ agonist was found to block or reverse the individual mPFC neuron studied. DAMGO attenuated the response to ACh and GLu in some cells, but in other neurons it clearly blocked the excitatory GLu response without altering the response to ACh. In a third group of neurons, DAMGO was found to have no effect on either the ACh- or GLu-induced response.

(Supported by NIDA DA05901 and NIDA DA08051)
726.17

The γ-aminobutyric acid (GABA) receptor agonist baclofen has prominent inhibitory actions in neuromodulatory regions of the mammalian CNS. For example, baclofen inhibits aromatic amino acid uptake by mouse hypothalamic neurons attributed to an increase in an inwardly rectifying K+ conductance. This response is identical to that following activation of µ-opioid receptors, which couples to the same effector in this brain region. The purpose of the present study was to characterize the pharmacology of the baclofen response, and to determine if cross-tolerance develops with µ-opioid agonists following chronic morphine treatment (subcutaneous implantation of pellets each containing 2mg of morphine) for 2-3 days each. To this end, intracellular recordings of acutely isolated neurons were made in coronal hypothalamic slices (60µm) prepared from ovarectomized female guinea pigs. At concentrations of 3mM, the GABA A receptor blockers CGP 37384 and 2-hydroxy-7-aminoclonidine shifted the baclofen dose-response curve to the right with an estimated K of 3.2±1.1µM (mean±SEM; N=3) and 15.9±4.2µM (n=2), respectively. Compared to placebo-treated controls, chronic morphine treatment reduced the potency of the µ-opioid receptor agonist DAMGO but not of baclofen. Taken together, these results show that: 1) the inhibitory effect of baclofen on acutely isolated neurons is consistent with its actions as a GABA A receptor agonist, and 2) chronic morphine exposure does not produce cross-tolerance between GABA A and µ-opioid receptor agonists. Further finding suggests that convergence of the two receptors systems to the same effector may be not a sufficient condition for cross-tolerance between receptor systems. (This work was supported by Grants DA05158 and DA07262).

726.19

We have previously reported that activation of µ opioid receptors inhibited NMDA-mediated synaptic currents in dentate granule cells. The present study further characterized this effect and examined possible involvement of the cAMP cascade. Synaptic currents were evoked by stimulating the dentate outer molecular layer, and recorded from granule cells using whole-cell voltage-clamp techniques in the hippocampal slice preparation. NMDA receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) were isolated in the presence of the AMPA antagonist DNQX and the GABA A antagonist bicuculline. Modulation of the amplitude of NMDA EPSCs was observed following bath application of the µ agonist PNU 101701 (0.1-10µM). This reduction seemed not dependent upon the presence of extracellular Mg2+, and could be effectively prevented by pertussis toxin included in the recording pipette. In contrast, the adenylyl cyclase activator forskolin (10-100µM) was found to increase the amplitude of NMDA EPSCs in a dose-dependent manner. Forskolin (100µM) also significantly attenuated PNU101701-induced reduction of NMDA currents. These results suggested that the cAMP cascade may be involved in µ opioid-induced inhibition of NMDA currents.

726.20
DEMONSTRATION OF µ-OPIOID RECEPTOR SUPPRESSION OF Ca2+ CURRENTS IN CENTRAL NEURONS. B.L. Soldo* and H.C. Moses. Department of Physiology, University of Michigan, Ann Arbor, MI 38109.

Opiates and opioid peptides have been shown to suppress voltage-sensitive Ca2+ currents in peripheral neurones of a variety of neuron-like cell lines; however, this effect has only recently been demonstrated in central neurons. In this study, whole-cell Ca2+ channel currents were recorded from acutely isolated neurones of rat ventral forebrain, a region rich in opiate receptors, to examine the possible coupling between opioid receptors and neuronal Ca2+ channels. High voltage-activated (HVA) currents were isolated by depolarizing steps (-10 mV to +10 mV) from a holding potential of -80 mV, using 4mm Ba2+ as charge carrier. Bath application of the µ-opioid selective agonist H-Tyr-D-Ala-Gly-Phe-NH2 (DAGO, 3µM) reversibly suppressed the peak amplitude of HVA currents (with 40% maximal effect) and slowed their rate of activation. The L- and N-type Ca2+ channel blockers, nifedipine, respectively, blocked pharmacologically distinct components of the HVA current in these neurones. The DAGO-sensitive component was reduced after blockade of N-type currents by GVIA. In addition, cells that allowed Ca2+ inhibitory responses to DAGO exhibited morphological features of principal neurones and stained positive for acetylcholinesterase. These data suggest that postsynaptic µ opioid receptors are negatively coupled to N-type Ca2+ channels in rat ventral forebrain cholinergic neurones. (Supported by NIH grant DA-03365).

727.1

5-HT1A receptors are involved in a lot of various physiological functions. 5-HT1 A receptors are principally localized postsynaptically in the limbic structures and in the raphe nucleus as serotonergic autoreceptors. With the aim of further analyzing the role of 5-HT1 A receptors in each of their precise localization, we have locally blocked their expression in the adult rat, using anti-sense oligonucleotides (Odns). Different Odns, complementary to the 5'-terminal portion of the mRNA coding for 5-HT1 AR, were designed as either unmodified, phosphorothioate or 2'-end-alkylamine-attached Odns. In order to validate this approach, stereotactic injections were performed in the dorsal hippocampus using mixtures of Odns (anti-sense, sense or scrambled) and lipofect. The mixtures were delivered either in single or repeated injections, or in a continuous mode using Alzet osmotic minipumps. The resulting effect on 5-HT1 A receptor density was analyzed by receptor quantitative autoradiography. Specificity and histological controls were performed. Experiments using single injections (2.5µg µ1) demonstrated that the 2'-end-alkylamine-attached Odns were the most efficient, giving up to 60% decrease of 5-HT1 A receptor density. The unmodified Odns were almost inefficient and phosphorothioate Odns exhibited cytotoxicity. Maximum effect was observed 4 days after injection.

Another approach using plasmids containing anti-sense RNAs is currently under investigation in order to obtain a continuous blockade for a longer period of time. Both approaches are used to block the expression of 5-HT1A autoreceptors in the raphe nucleus to assess their specific role in anxiety.

727.2
IN VITRO AND IN VIVO ALKYLATION OF CENTRAL 5-HT1A RECEPTORS. E. Radi*, F.K. Neunert, M. Carl and T.A. Reader. CRSN. Département de physiologie, Faculté de médecine, Université de Montréal, (Qué) Canada.

Saturation binding of the serotonin agonist [3H]-OH-DPAT in cerebral cortex and hippocampus is best fitted to a two-site model, indicating an heterogeneity of receptors or several binding affinity states. For cerebral cortex the affinities are of about 0.7 nM and 36 nM, and for hippocampus of 0.8 nM and 22 nM. Also, binding of 1 nM [3H]-OH-DPAT is inhibited by several 5-HT1A antagonists and agonists and, except for ritanserin, all competition curves are best described by a two-site model. The in vivo treatment of the membranes with N-ethylmaleimide (NEM) to alkylate sulfhydryl groups causes dose-dependent decreases of [3H]-OH-DPAT binding; all the inhibition curves are biphasic and the efficous irreversible. After in vivo treatments, carried out by treating rats with N-ethylcarboxamido-1-2-ethoxy-1,2-dihydroquinoline (EEDQ; 10 mg/Kg i.p.), the saturation curves from both control and EEDQ-treated rats are best fitted to a two-site model. However, for EEDQ-treated animals there is a drastic decrease in 5-HT1A receptors that were resistant to hippocampal but not in cerebral cortex. In contrast, the low-affinity binding sites remained unchanged, indicating that this site is not a G protein coupled receptor. Since the decrease in 5-HT1A receptors is not produced in an individual basis, the results suggest independent regulations of the two [3H]-OH-DPAT binding sites. Altogether, the present data further supports the concept that [3H]-OH-DPAT, besides labelling 5-HT1A receptors, also binds to other sites in rat cerebral cortex and hippocampus. (Supported by the MRC(C) and the Savory Foundation)
7.7.3
NCS-MPP (4-(2'-methoxy-phenyl)-1'-2'-(2'-pyridyl) p-isothiocyanatoethyl-piperazine) is a high affinity and irreversibly binding ligand for 5-HT1A receptors.

7.7.4
NCS-MPP is a (4-(2'-methoxy-phenyl)-1'-(2'-pyridyl) p-isothiocyanatoethyl-piperazine), which binds irreversibly to serotonin (5-HT) receptors and the binding is irreversible.

7.7.5

7.7.6

7.7.7
Quantitative autoradiographic analysis of 5-HT1A receptors using agonist and antagonist radioligand.


7.7.8
DISTRIBUTION OF GABA AND 5-HT IMMUNOCOMPLEXES IN THE RAT HIPPOCAMPUS, J.D. Fantz and J.C. Davis, Medical Neurochemistry, and Dept. of Anatom. Indiana Univ. School of Med., Indianapolis, INDIANAPOLIS.

GABA and serotonin (5-HT) are known to be co-localized in specific areas of the brain. GABA is an inhibitory neurotransmitter that acts at GABA receptor sites, while serotonin is a neurotransmitter that affects mood, sleep, and appetite.

7.7.9
AGE-RELATED ALTERATIONS IN SEROTONIN RECEPTOR FUNCTION IN FOREBRAIN REGIONS FOLLOWING EDEQ TREATMENT. J.B. Kost* and J.M. Lakoski. Departments of Pharmacology & Anesthesia, Pennsylvania State University College of Medicine. Hershey, PA 17033.

EDEQ (1-ethylcyclohexane-1-ethyl-d2-1,2,3,4-tetrahydouracil) is a neurotoxin which binds irreversibly to serotonin (5-HT) receptors and when used as a tool for studying receptor function.

7.7.10
24 hr prior to sacrifice were used to examine changes in 5-HT receptor function with age. Scattered analyses using the 5-HT1A binding sites were conducted in forebrain regions which contain high densities of this receptor subtype.

A significant decline in [3H]-OH-DPAT binding in hippocampal tissue was demonstrated in EDEQ treated rats, with age-related changes in the binding capacity of 5-HT receptors. This suggests that the age-related changes in 5-HT receptor function are due to a decrease in the number of 5-HT receptors.
727.10 DIFFERENTIAL EXPRESSION OF SEROTONIN RECEPTORS 5-HT1A AND 5-HT2A mRNA IN VASCULAR CELLS AND ASTROCYTES IN CULTURE. Z. Cougher, I. Bouchelet, W.Y. Yang, J. Hulme, S. Stuginski, and E. Haimo. Centre Neuro-Cellulaire des Tissus, University of Montreal, Montreal, Canada. Supported by an operating grant from the Medical Research Council of Canada, Ottawa, ON, Canada.

Serotonergic receptors are also expressed in vascular smooth muscle cells (SMCs) and astrocytes (Acs) of the brain and peripheral organs. However, their expression and function in these cell types is not well understood. Here, we examined the differential expression of 5-HT1A and 5-HT2A mRNA in SMCs and Acs isolated from rats by reverse transcription (RT)-PCR using species-specific oligonucleotide primers. The effects of 5-HT on 5HT1A and 5HT2A mRNA expression in SMCs and Acs were compared with those in the brain under identical conditions. The expression of 5HT1A mRNA in SMCs and Acs was significantly higher than in the brain, whereas the expression of 5HT2A mRNA was similar in all three tissues. These findings suggest that serotonergic receptors may play a role in the regulation of vasomotor function and cerebral vascular tone.


Changes in rat brain and spinal cord 5-HT1A receptors following 5,7-DHT treatment were examined using an agonist [32P]-OIH-PITAP (PITAP) as well as a newly developed antagonist ligand [125I]-p-MPPI (MPPI). Adjacent coronal sections of control and 5,7-DHT-treated rat brains (n=14/group) and spinal cords (n=20/group) were labeled with both ligand and binding was measured with the use of quantitative autoradiography. (1) Levels of MPPI binding were higher in hippocampal subfields compared to PITAP binding, whereas, in cortical layers PITAP binding levels were higher. (2) Agonist (PITAP) binding to hippocampal or cortical regions of 5,7-DHT-treated animals was similar to controls, as has been previously observed. (3) Antagonist (MPPI) binding in hippocampal or cortical regions of 5,7-DHT-treated animals was also unaffected compared to controls, except in the CA1 subfield of the hippocampus. (4) In the cervical but not the lumbar portion of the spinal cord, 5,7-DHT-treated rats showed increased levels of MPPI binding in the most superficial layer of the dorsal horn. In conclusion, the lack of changes in 5-HT1A agonist binding in rat brain following 5,7-DHT treatment has been extended to antagonist binding, except in the CA1 region of the hippocampus. Regional differences in the ratio of agonist to antagonist binding suggest regional differences in the coupling of 5-HT1A receptors to G-proteins. (Supported by MH-48125, MH-43821)
727.15

Serotonin Receptors: 5-HT₃ And Human SHT₂ receptors.

727.16

Pharmacological activity of solubilized human SHT₁a receptor.

727.17

Interactions of (S)-4-UH-301 derivatives with recombinant 5-HT₁a receptors.

727.18

Way 100635 reverses the decrease of 5-HT levels produced by the putative 5-HT₄ antagonist, WAY 101355.

727.19

Direct activation by dopamine of recombinant human 5-HT₄ receptors expressed in Xenopus oocytes.

727.20

Efficacy of antipsychotic drugs at cloned human serotonin (5-HT₄) and dopamine (D₁) receptors, determined by stimulation of [³H]gtp binding.

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Society for Neuroscience, Volume 21, 1995
1H]5-HT15535, a NOVEL, SELECTIVE RADIOIDOLIGAND AT SEROTONIN (5-HT1A) RECEPTORS: CHARACTERISATION OF BINDING TO CLONED HUMAN AND RAT HIPPOCAMPAL 5-HT1A RECEPTORS. J.E. Pierson*, A. Newman-Tancrosi, I. Verticchi and M.I. Millan, Instituto de Investigaciones Servier, 125 Chemin des Ronde, 78290 Croissy, Paris, France.

The novel benzodiazepine receptor. 5-HT15535 (4-benzodioxan-5-yl-indan-2-yl)piperazine) is a highly selective ligand for serotonin 5-HT1A receptors (Millan et al., I.F.E.T., 268, 337-352, 1994). The compound was isolated (8 Columns) and its binding profile characterised in CHO cells stably expressing the human 5-HT1A (5-HT1A) receptor. At 22 °C, [1H]5-HT15535 associated to 5-HT1A receptors with a half-life of 3.4 minutes. In saturation binding experiments, [1H]5-HT15535 displayed a Kd (1.3 nM) and a Bmax (14 pmol/mg) similar to that observed in this cell line with the prototypic 5HT1A agonist, [1H](-)-8-OH-DPAT (1.0 nM and 1.1 pmol). The Kd for S 15535 agreed well with the Kd value derived from competition of unlabeled S 15535 with [1H]-OH-DPAT (0.8 nM) in competition binding experiments with [1H]-5-HT15535. serotoninergic ligands, including (-)-8-OH-DPAT, 5-HT, (+)-WAY 100,135 and spiperone, displayed Kd's consistent with those observed with [1H]-3H-OH-DPAT, with values of 1.2, 1.7, 14 and 460 nM respectively. GppNHp (100 μM) only slightly reduced the binding of 5-HT 15535 by 18 % as compared to that of [1H]-OH-DPAT (70 %) to 5-HT1A receptors. Further, ATP (100 μM) also inhibited (-10 %) [1H]5-HT1555 binding. [1H]5-HT1555 showed high affinity, saturable binding to rat hippocampal membranes (Kd = 2.4 μM, Bmax = 820 fmol/mg) although its association rate was slower than that of 5-HT1A. As for 5-HT1A receptors, the binding of [1H]5-HT15535 to hippocampal membranes was slightly reduced (-10 %) by GppNHp (100 μM). These results suggest that [1H]5-HT1555 exerts agonist (or weak partial agonist) properties at both human and rat 5-HT1A receptors for which it represents a novel, useful radioligand.

T27.22


The ventral pallidum (VP) of the basal forebrain contains high levels of serotonin (5-HT). In addition, 5-HT3 receptors exist on the soma of VP neurons and 5-HT4 receptors are found on the pallidal terminals of striatal projection neurons. The present study compares the effects of the 5-HT4 agonist I-38-OH-DPAT (1.296 μg/kg i.v.) and the 5-HT3 agonist CP-94253 (0.01-6.4 μg/kg i.v.) on the non-selective 5-HT4 agonist TFMPP (0.01-2.9 μg/kg i.v.) on single-neuron activity in the VP of chloral hydrate-anesthetized rats. TFMPP dose-dependently reduced the firing rate of 100% of VP neurons tested (n = 7). In contrast, 8-OH-DPAT produced dose-dependent increases (n = 7) and decreases (n = 4) in VP activity. CP-94253 had no effect on VP neurons and produced only transient and/or variable changes in the activity of 7 additional neurons. Within the latter group, 3 neurons decreased firing rate but these responses were not antagonized by the 5-HT4 antagonist (-)lindopil (0.1-0.2 μg/kg). These results suggest that the alterations in VP neuronal activity produced by TFMPP were not mediated by 5-HT4 receptors. While 5-HT3 receptor activation decreased the firing rate of some VP neurons, other 5-HT4 receptor subtypes are also likely to contribute to the effects of the TFMPP on VP activity.

Supported by USPHS grant MH51810 to TCN. CP-94253 was generously provided by Pfizer Central Research, Groton, CT, U.S.A.

T27.23

SEROTONIN INHIBITS NUCLEUS ACCUMBENS NEURONS RECEIVING INPUTS FROM PARAFASCICULAR NUCLEI OF THALAMUS VIA 5-HT1A RECEPTORS BUT NOT THOSE FROM HIPPOCAMPUS. N.A. Nagarka*, A. Takai, M. Sasa*, S. Yamawaki*. Departments of Psychiatry and Neurosciences and Pharmacology Hiroshima University School of Medicine, Minami-ku, Hiroshima, 734, Japan.

The nucleus accumbens (Acc) neurons receive innervation from various areas of the brain such as parafascicular nucleus of the VP (PF), hippocampus, amygdala and ventral tegmental area. Serotonin (5-HT) nerve terminals and 5-HT receptor subtypes (5-HT1A, 5-HT2B, 5-HT3C, 5-HT2 and 5-HT3) receptors) are found in Acc. However, the role of 5-HT receptors in Acc neurons functionally remains unknown. Therefore, microiontophoretic studies were performed to elucidate the role of 5-HT1A receptors of Acc neurons receiving inputs from Pf and hippocampus (HPC). Single neuronal activities were recorded with a glass microelectrode along with a second-barreled microelectrode in chloral hydrate-anesthetised rats. Each of the barrels was filled with dopamine (DA), 5-HT, 8-OH-DPAT5-HT1A agonist), NAN-190 (5-HT1A antagonists), nardoatin, glaucoma receptor, and neurons were recorded for the immediate vicinity of the target neuron recorded using by microiontophoresis method. Spikes elicited by the Pt stimulation were inhibited by iontophoretically applied DA or 5-HT and 8-OH-DPAT in a dose-dependent manner. Glutamate-induced firing as well as by simple synaptic addition of DA, 5-HT or 8-OH-DPAT. 8-OH-DPAT induced inhibition of glutamate-induced firing was antagonized drug administration of NAN-190. However, spikes elicited by HPC stimulation were not affected by iontophoretically applied DA or 5-HT. These results suggest that the DA-sensitive Acc neurons receiving inputs from Pt are inhibited by 5-HT via 5-HT1A receptors but those from hippocampus are not affected.

T27.24

EFFECTS OF 8-OH-DPAT ON RELEASE OF CORTICOTROPIN RELEASE FACTOR IN AMYGDALE. E.A. Johnston*, V. Gardaghi and D.L. Beela, Depts. of Behavioral Medicine and Psychiatry, Neurology, Pharmacology and Toxicology, West Virginia University School of Medicine, Morgantown, WV 26506.

Corticotropin Releasing Factor (CRF) is a 41 amino acid peptide neurotransmitter with putative roles in stress and anxiety. Release of CRF from the hypothalamus results in increased release of ACTH from the anterior pituitary, ultimately leading to glucocorticoid release in the stress response. Increasing evidence indicates that release of CRF from sites in the central nervous system, other than the hypothalamus, may play a role in anxiety. The amygdala is a brain region critical for the expression of amygdala functions as a modulator of anxiety. The amygdala is innervated by serotonergic neurons originating in the dorsal and medial raphe nuclei. Amygdala neurons are activated in vivo by 8-OH-DPAT (0.01-100 nM) under basal and depolarizing conditions. 8-OH-DPAT caused no change in basal CRF release but dose dependently increased potassium-induced release of CRF from amygdala slices (p < 0.05). The maximal effect was observed using 1 μM 8-OH-DPAT which is consistent with the affinity reported for 8-OH-DPAT binding to 5-HT1A receptors (0.7-2.0 μM). These results are consistent with previous reports of the stimulatory action of 8-OH-DPAT on hypothalamic release of CRF.

This work was supported by the Bethesda Research Endowment at WVU School of Medicine (EAS) and by NSF grant 9522293 (DLB).
728.1  
THE 5-HT5 RECEPTORS: CHARACTERIZATION OF THE HUMAN 5-HT5A RECEPTOR. ABSENCE OF THE HUMAN 5-HT5B RECEPTOR.  
KNOCKOUT OF THE MOUSE 5-HT5A RECEPTOR.  
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We have isolated from a human genomic library, the homologues of the mouse 5-HT5A and 5-HT5B genes. The human 5-HT5A gene encodes a protein with similar characteristics to the mouse 5-HT5b receptor. The human 5-HT5A receptor is expressed in Cns-2 cells, the human 5-HT5-receptor displayed a high affinity for the radiolabelled ligand [5-HT5CT (Kd=2.68M)] and [5-HT5B (Kd=1.72M)]. These sites were insensitive to Gpp(NH)P and display a similar pharmacological profile as the mouse 5-HT5 receptor. We are currently analysing the coupling of the human 5-HT5A receptor in NIH3T3, 293 and L540 cells. Receptor-PCR experiments revealed expression of the human 5-HT5a mRNA in cortex, hypophysis, amygdala, thalamus, raphe nuclei and spinal cord.  

The human 5-HT5B gene is composed of two exons and is transcribed at very low level. However, unlike the 5-HT4a gene, the 5-HT5B gene does not appear to encode a functional protein, since the putative coding sequence is interrupted by several stop codons. In addition, Southern and PCR experiments performed on samples from different individuals, have revealed that this non-functional gene is the only human homolog of the rodent SHT5B gene. These results indicate that humans do not express a functional 5-HT6 receptor and suggest that this protein has been lost after the divergence between rodents and primates.  

In order to study the function of the 5-HT5A receptor, we have generated by homologous recombination, mutant mice lacking the gene encoding this receptor. These homozygous mutants develop, move, feed and breed apparently normally. We are currently investigating their behavior in various experimental paradigms as well as their response to drugs that have a high affinity for the 5-HT5A receptor, such as LSD.

728.3  
5-HT, RECEPTORS IN RAT BRAIN: mRNA AND RECEPTOR DISTRIBUTIONS  
SUGGEST BOTH SOMATODENDRITIC AND PRESYNAPTIC LOCALIZATIONS.  
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Oligonucleotide probes and in situ hybridizations were used to study the distribution of mRNA encoding the long (5-HT1C) and short (5-HT1A) splice variants of the serotonin 5-HT1 receptor. With a probe common to both variants, the strongest hybridization was detected in the striatum, hypothalamus (VTA), olfactory bulbs, caudate (ICj) and medial habenula (MhB). Strong signals were observed in nucleus accumbens (Acb) and caudate-putamen (Cul), this latter showing a rostro-caudal increasing gradient. Intermediate and low levels were seen, respectively, in the granular cell layer of the dentate gyrus and in the pyramidal cell layer of Ammon's horn. With a probe specific for 5-HT1A, essentially the same pattern was observed. Finally, a probe specific for 5-HT1D receptor showed an expression pattern of distribution, with low levels of signal in ICj, MhB, Acc and caudal parts of CPu and very low levels in dentate gyrus.  

5-HT1D receptor binding sites were visualized with the 5-HT1a antagonist [3H]5-HT 207710. The highest densities of receptor were observed in OTu, Acc, CPu, globus pallidus (GP), substantia nigra (SN) and interpeduncular nucleus (IP). Lower densities were present in medial and lateral habenula and hypothalamus, among many others. Comparison of mRNA and receptor distributions suggests two different subcellular localizations for 5-HT1 receptors. Receptors present e.g. in OTu, Acc, CPu and hippocampus would be located somatodendritically, whereas receptors present e.g. in GP, SN and IP would be located pre-synaptically on axon terminals of, respectively, the striatopallidal, strio-arcuate, and habenulo-interpeduncular projections.  

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728.5  
5-HT4 RECEPTOR LEVELS ARE REDUCED IN THE PUTAMEN FROM PATIENTS WITH SCHIZOPHRENIA.  
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Relatively high densities of the 5-HT4 receptors are expressed in human mesolimbic and nigrostriatal systems, as detected using the high affinity and selective radioligand [3H]113808. Our recent data in rat brain indicate that the 5-HT4 receptor can modulate the release of dopamine. It has been generally accepted that an overactivity of the mesolimbic dopamine system is associated with schizophrenia. Therefore, in the present studies we investigated the levels of 5-HT4 receptors in human brain regions from schizophrenic patients and matched control patients who had died without a neurological or psychosocial disorder.  

Saturated binding experiments with [3H]113808, revealed specific binding (non-specific defined by SD2 205-577, 10 mA) in all examined tissues from control and schizophrenic patients (putamen, amygdala, nucleus accumbens and substantia nigra). These results indicated a significant (p < 0.05) reduction in [3H]113808 labelled 5-HT4 receptor density in putamen tissue from schizophrenic patients when compared to control putamen tissue (approximately 50 %). Whilst no significant differences in the densities of the radiolabelled 5-HT4 receptor were detected in amygdala, nucleus accumbens and substantia nigra from schizophrenic and control patients.  

5-HT4 receptor density in human putamen of schizophrenic patients, is part of the pathology of the psychiatric disorder or a consequence of chronic psychotropic drug therapy, remains to be established. We would like to thank the MRC Brain Bank (Cambridge, UK) for the donation of tissue.

728.6  
ABILITY OF THE 5-HYDROXYTRYPTAMINE 4 RECEPTOR TO MODULATE DOPAMINE AND 5-HYDROXYTRYPTAMINE RELEASE IN RAT BRAIN.  
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In the present study we assess the ability of the 5-hydroxytryptamine (5-HT4) receptor to modulate dopamine (DA) and 5-hydroxytryptamine (5-HT) release in the rat forebrain. The 5-HT4 receptor agonist ondansetron enhanced dopamine release from rat urinal slices and in the striatum but not in the substantia nigra.  

A significant (p < 0.05) reduction in [3H]113808 labelled 5-HT4 receptor density in putamen tissue from schizophrenic patients when compared to control putamen tissue (approximately 50 %). Whilst no significant differences in the densities of the radiolabelled 5-HT4 receptor were detected in amygdala, nucleus accumbens and substantia nigra from schizophrenic and control patients.  

5-HT4 receptor density in human putamen of schizophrenic patients, is part of the pathology of the psychiatric disorder or a consequence of chronic psychotropic drug therapy, remains to be established. We would like to thank the MRC Brain Bank (Cambridge, UK) for the donation of tissue.
Co-localization of serotonin 5-HT5 and 5-HT2C receptors in neuroepithelial containing neurons of the rat striatum

**728.7**

Co-localization of serotonin 5-HT5 and 5-HT2C receptors in neuroepithelial containing neurons of the rat striatum. J.P. Ward* and D.M. Davis, Dept. of Pharmacology and Dept. of Psychiatry, University of Washington, Seattle, WA 98195.

The striatum is the largest nucleus of the basal ganglia, and has two main output: a projection to the globus pallidus, and a projection to the substantia nigra. Dopamine receptors and certain neuropeptides which are expressed in the striatum are differentially distributed between these two pathways. Specifically, enkephalin and the dopamine D2 receptor are expressed in neurons projecting to the globus pallidus, while substances P and POMC and the dopamine D1 receptor are expressed in neurons projecting to the substantia nigra. The striatum also receives a prominent serotonergic input, but little is known about how serotonin receptors fit into the molecular neuroanatomy described above. In the present study we used double label in situ hybridization to determine the distribution of the mRNAs of two of the serotonin receptors, the 5-HT5 and the 5-HT2C, in relation to enkephalin, substance P, and dynorphin expressing neurons.

Regional sections of rat brain were simultaneously hybridized with an 35S riboprobe for one of the serotonin receptors and a digoxigenin labeled riboprobe for one of the neuropeptides. Sections were examined using brightfield microscopy, and the relationship of expression of the two mRNAs to each other was determined. mRNAs for the 5-HT5 receptors was found to be evenly distributed throughout the striatum and nucleus accumbens and to co-localize with all three of the neuropeptides examined. This indicates that signals mediated via this receptor will affect both of the major output pathways.

The 5-HT2C receptor also showed co-localization with all three of the neuropeptides examined, however its distribution is considerably more complex. Levels of 5HT-2C mRNA were much higher medially than laterally, and ventrally than dorsally. Additionally discrete regions found throughout the striatum exhibited much higher levels of expression. These small scattered regions represent the striatal patch compartment, as shown by their overlap with regions which exhibit high levels of dynorphin mRNA expression. Supported by NS20111 and a F.M.A. Fellowship.

**728.9**

Effects of clozapine and haloperidol on expression of 5HT5 and 5HT7 receptors: J.A. Frederick, J.F. Lopez* and J.H. Meador-Woodruff, Mental Health & Geriatrics, Institute of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

The distribution and regulation of messenger RNA encoding two recently cloned serotonin receptors was examined by in situ hybridization in the rat brain. 5-HT7 labelling was observed in the striatum, olfactory tuberce, neocortex, and hippocampus. 5-HT5 labelling was observed in the thalamus, hypothalamus, piriform cortex, entorhinal cortex, superficial layers of neocortex, septum, amygdala, and the CA2 and CA3 regions of the hippocampus. Most striking was the lack of 5-HT7 expression in the dentate gyrus or CA1 regions of hippocampus, or in the striatum. The high affinity of 5-HT5 and 5-HT7 receptors for atypical antipsychotic drugs, and their localization in limbic and cortical regions, suggest that they may play a role in the pathophysiology of schizophrenic disease. In addition, expression of these receptors is regulated by antipsychotic drugs; rats were injected with clozapine (20 mg/kg/day, N=8), haloperidol (2 mg/kg/day, N=8), or vehicle (N=8) for two weeks. Preliminary evidence suggests that clozapine and haloperidol increase expression of 5-HT7 receptors in the nucleus accumbens, and decrease 5-HT5 expression in frontal cortex. Meanwhile, no differences in 5-HT5 expression were observed between clozapine- or haloperidol-treated animals and all animals studied. Further results of this study are expected and will be presented.

**728.10**


We have used receptor autoradiography with [3H]-carboxytryptamine (H-5-CT) as ligand and in situ hybridization histochemistry using [3H]-labeled oligonucleotides to visualize the anatomical localization of the 5-HT5 receptor and its mRNA in the rat and guinea pig brain. By analyzing the displacement of 5 nM [3H]-5-CT binding by the selective 5-HT5 receptor antagonist WAY 101355 and 5-HT5 antagonists WAY 101353 and 5-HT1A receptor agonist, 8-OH-DPAT, a 5-HT5, and H5-HT7 conditions were set and used for the direct visualization of 5-HT, binding sites. In the guinea pig, 5-HT7 receptors could be selectively labeled with 5 nM [3H]-5-CT in the presence of high concentrations of WAY 101353 (100 nM) and WAY 101355 (100 nM), whereas in the rat, a small population of 5-HT5, receptors remained still labeled.

In these conditions, in the guinea pig 5-HT, binding sites were found to be enriched in layers III of the neocortex, caudatum, several hypothalamic and midline nuclei of the thalamus (e.g. paraventricular, paracentral, parietal and medial thalamic nuclei), diencephalon and brain stem. These areas were also enriched in 5-HT, mRNA signal, both in rat and guinea pig. In contrast, neither 5-HT, binding nor mRNA were detected in structures such as the globus pallidus or the par compacta and reticulata of the substantia nigra.

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**729.1**


Thioperamide is the first selective high affinity antagonist described for the H3 receptor. Its use as a radioligand has, so far, never been reported. In the present study, the binding of [3H]-thioperoxidase was characterized in membranes of rat brain cortex. Transformation of saturation curves showed non-linear Scatchard plots suggesting labelling of two different populations of H3 receptors ([3H]-thioperoxidase).

A maximum concentration of 1 mM, H3 agonists hardly displaced the total binding of 5 nM [3H]-thioperoxidase. Moreover, although H3 antagonists fully displaced the total binding of 5 nM [3H]-thioperoxidase, K0.5 obtained were different from the values expected for the H3 receptor. These results showed that, at 5 nM, [3H]-thioperoxidase was mainly labelling a non-H3 receptor site. The total binding of 0.3 nM [3H]-thioperoxidase, however, was, in contrast, up to 60% by H3 agonists. Stereospecificity for the R and S isomers of a methylated histamine derivative for the displacement assay was obtained by [3H]-methylmephistamine was established. H3 antagonists fully displaced the binding of 0.3 nM [3H]-thioperoxidase. Displacement curves of the antagonists were biphasic for the high affinity site similar to values expected for the H3 receptor. In conclusion, in rat brain, [3H]-thioperoxidase, labels with high affinity both the H3 receptor and a unidentified H3-like receptor. H3 antagonists also show high affinity for the non-H3 site. Because antagonists do not show high affinity for the 5-HT2C [3H]-thioperoxidase, they should be used to define the non-specific binding of [3H]-thioperoxidase when studying the H3 receptor.

**729.2**


There is extensive evidence from early studies that histamine is not, if exclusively methylated in mammalian brain. However, we recently showed that imidazoleacetic acid (IAA), histamine's oxidative metabolite in the periphery and a potential brain agonist, is present in brain in levels which increase when histamine methylation is blocked. IAA does not enter brain from periphery. H3-histamine (lev) formed H3-IAA and its ribosylated metabolites in rat brain, a process that increased when histamine methylation was blocked (J. Neurochem. 65 [in press]). To assess which enzyme(s) may be involved, we examined metabolism of 2-10 ng H3-histamine in whole brain homogenates of male rats, mice and guinea pigs (2 mg; 5 ml; NaP buffer, pH 7.4) incubated (37°C) with ammonia oxidase inhibitors (e.g. clorglyaine, denepry or aminoguanidine) [AG], then boiled after 30 min. Aliquots (5 ul) were applied to polygram silica TLC plates (250 ml) and separated histamine (Rg 0.3), pyridylmethylhistamine (0.14), pyridylmethylimidazoleacetic acid (0.52) of IAA and 16S in all species examined, H3-IAA (and/or its metabolites) was produced. Clorglyaine and denepry, at conces selective for M-A and -B, respectively, did not affect recoveries of H3-histamine or H3-IAA (and its metabolites). In contrast, at conces expected to abolish DAO activity (up to 1 μM), AG inhibited, but did not abolish oxidation. These results confirm that histamine is not oxidized by MAO-A or -B and suggest that an Aminergic enzyme, possibly DAO or another amine oxidase(s), can oxidize histamine in brain and form IAA and its metabolites. (NINDS-NS-28012)

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729.3


The release of histamine (HA) in the CNS is under the negative tonic control of presynaptic histamine H1 receptors. HA release is metabolized to N′-methylhistamine (TMH). Selective HA receptor antagonists block the negative feedback of HA, resulting in increased HA release and metabolism. GT-2016, a non-competitive histamine H1 receptor antagonist, has been described as an effective Glaehle. In vivo penetration of GT-2016 and changes in HA turnover were studied in male Sprague-Dawley rats, and compared to the thio- and isothiourea-containing H1 antagonists, thioperamide, and thiopropylamine. The in vivo Ks for GT-2016, thiopropylamine, and diopropylamine were 4.4, 1.0, and 0.2 nM, respectively, using rat cortical membranes and 11H-N′-methylhistamine (NAMHA). The ability of these compounds to penetrate the blood-brain barrier was estimated using an ex vivo binding technique. Rats were pretreated with drug (1 hr) and an inhibition binding assay was performed using unlabeled cortical homogenates. Each of the drugs (1 to 30 mg/kg, i.p.) produced a dose-dependent inhibition of NAMHA binding. The CNS bioavailability of GT-2016 was 10 to 15 fold greater than that of thiopropylamine and approximately 150 fold greater than the CNS bioavailability of diopropylamine. Clobenpropit produced minimal changes in HA and TMH levels. GT-2016 and thiopropylamine produced dose-dependent increases in the TMH/HA ratio, indicative of increased HA release and metabolism. These increases in the TMH/HA ratio were directly correlated with the degree of receptor binding shown in the ex vivo binding studies. In summary, GT-2016, a novel non-competitive HA antagonist, provides good CNS bioavailability and correspondingly functional blockade of CNS H1 receptors.

729.5


Structural and biochemical interactions between arachnoid cells from the basis of the cerebrospinal fluid (CSF)-blood barrier. Using cultured porcine spinal arachnoid cells, we have begun to assess the types of receptor/second messenger systems expressed by arachnoid cells that may be involved in the regulating the CSF-histamine turnover. Activation of phospholipase C (PLC) was measured in confluent arachnoid cells by monitoring the conversion of 1H-myoinositol to 1H-inositol phosphates. Adenylyl cyclase (AC) activity was measured by preincubating the cells in 1H-adenosine to form 1H-ATP then measuring the conversion of 1H-ATP into 1H-cyclic AMP. Porcine spinal arachnoid cells in vitro expressed both PLC and AC activity. Specifically, bovine adenylyl and histamine stimulated PLC at EC50 of 34 µM and 11 µM, respectively. The possibility of carbobol was inhibited with atropine. The histamine effect was inhibited by H1-receptor antagonists, but not by H2- or H3-receptor antagonists. As stimulated by forskolin and prostatin and P2D2 (P2D2). Histamine did not stimulate AC activity, indicating that H2-receptors were not present on the cells. In conclusion, porcine arachnoid cells in culture express muscarinic and histamine H1-receptors linked to PLC, and P2D2-receptors linked to AC. These receptors may be involved in regulating arachnoid cell dependent permeability of the CSF-blood barrier. Supported by a Burroughs Welcome Osteopathic Fellowship and a grant from the National Multiple Sclerosis Society.

729.6

CRANECTOMY ACTIVATES DURAL MAST CELLS AND INCREASES CEREBRAL CORTICAL HISTAMINE. Edward L. Anderson* and Martha E. Stolzley. Department of Anatomy and Cell Biology, U.N.T. Health Science Center, Fort Worth, TX 76107.

The dura mater contains numerous mast cells which are degranulated in response to various types of head trauma such as cryogenic lesions (Orr, 1988, Neurochem. Pathol. 8:43-51) or simple craniectomy (Olesen 1987, Acta Physiol. Scand. 130:63-66. Since degranulated mast cells release large quantities of histamine (HA) and histamine, we have investigated if dural mast cells can alter the diameter and permeability of pial blood vessels (Yong, et al., 1994, J. Neurotrauma, 11:161-171), the possibility exists that histamine from dural mast cells may cross the meninges to enter the subarachnoid space and affect adjacent brain tissue and pial blood vessels. To test this possibility, adult female Lewis rats were anesthetized by inhalation of isoflurane, subjected to unilateral craniectomies, then killed 10 min. later. Samples of cerebral cortex and meninges subjacent and contralateral to the craniectomies were assayed for HA using a specific radioenzymatic assay. Compared to contralateral tissues, the meningeal HA concentration was 62.7 ± 11.8% (mean ± SEM, n=5) of control, while the subcortical cerebral cortical HA concentration was 452.5 ± 16.5% (mean ± SEM, n=5) of control. Since the concentration of HA in the meninges (2.13 ng/mg wet weight) is 140-fold higher than the concentration in the cerebral cortex (0.015 ng/mg wet weight), the decrease in dural HA can easily account for the increased cortical HA observed on the lesioned side of the head. These results suggest that HA, and possibly other products of dural mast cells can cross the meningeal barrier to enter the subarachnoid space and affect underlying brain and associated tissues. (Supported by a grant from the National Multiple Sclerosis Society.)

729.7

STIMULATORY EFFECT OF HISTAMINE ON CALCIUM EFFLUX FROM CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. H. Houshi, K. Kira, M. Minakuchi, Y. Ishimura, M. Okuno, T. Ouchi and M. Ohno. Department of Pharmacology, Tokushima University School of Medicine, Kurumoto, Tokushima, 770, Japan.

Histamine is known to have several effects on adrenal chromaffin cells mediated by its H3 and H2 receptors. The histamine H3 receptor is associated with secretion of catecholamine, accumulation of inositol phosphates, increase in the intracellular level of free Ca2+ ([Ca2+]i), synthesis of opioid peptides, and phosphorylation of tyrosine hydroxylase in bovine adrenal chromaffin cells. On the other hand, the histamine H2 receptor is associated with accumulation of cyclic AMP in these cells.

In this study, the effect of stimulation of the histamine receptor on Ca2+ mobilization in cultured bovine adrenal chromaffin cells was examined. Histamine (10-7 M) increased [Ca2+]i to a peak in the presence or absence of extracellular Ca2+, followed by decrease with time. Histamine (10-7 to 10-5 M) also stimulated 45Ca2+ efflux from cultured bovine adrenal chromaffin cells in a concentration dependent manner. Its stimulatory effect on 45Ca2+ efflux was inhibited by the specific histamine H1 receptor antagonist mecamylamine. The increase in histamine-stimulated 45Ca2+ efflux was inhibited by NA* and by the Na+/Ca2+ exchange inhibitor amiloride. In addition, histamine stimulated 22Na+ influx into the cells, and this action was inhibited by amiloride.

These results suggest that stimulation H1 receptor induces extracellular Na+-dependent Ca2+ efflux from cultured bovine adrenal chromaffin cells, probably activation of Na+/Ca2+ exchange.

729.8

DETERMINATION OF AGONIST OR ANTAGONIST ACTIVITY AT THE MELATONIN RECEPTOR BY AN AFFINITY SHIFT METHOD. A. M. Lovelace, R. F. Brunst*, Eli Lilly and Company, Indianapolis, IN 46285.

Receptors exist in resting and activated conformations. Agonists bind preferentially to the activated state. In the present study, we investigated whether this phenomenon can be used to distinguish agonists from antagonists at the melatonin receptor. Binding of [125I]iodomelatonin to chicken brain membranes was measured in the presence of 10 mM MgCl2 (activated state) and 1 mM EDTA, 100 µM GTP, and 150 mM NaCl (resting state). The Ki of melatonin under activated conditions was 0.22 nM and under resting conditions was 1.70 nM, a 7.04 fold shift in affinity. In contrast, the antagonist luzindole had an activated state Ki of 0.79 nM and a resting state Ki of 0.80 nM, a shift of 1.01 fold. This confirms that the affinity shift method can distinguish melatonin receptor agonists from antagonists.
the synaptic vesicle glutamate uptake system: reconstitution with ATPAse and a partially purified glutamate transporter. S. M. Lewis, J. T. Uchida.

Molecular characterization and developmental expression of a murine high-affinity glutamate transporter. M. Sutherland, T. A. Dalman, L. J. Rothberg.

Postsynaptic uptake of excitatory amino acids in rat cerebellar Purkinje cells. T. Kataoka, H. Ishibori, and K. Kataoka.


Glutamate uptake activity in brain regions thought to play a role in the induction and propagation of seizures. Theoretically, this phenomenon could be mediated by modulation of high-affinity uptake systems for glutamate. To date, three distinct glutamate transporters have been cloned: GLT-1, GLAST, and EAAC-1. Immunocytochemistry studies have shown that GLT-1 and GLAST are localized to glia, whereas EAAC-1 is localized to neurons. We investigated whether there were changes in the levels of these transporters in rats that had undergone kindling-induced seizures by stimulation of the amygdala.

Glutamate uptake inhibitors: the potential for preclinical evaluation. The PKM is the recipient of a PNNCA predoctoral award.

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A number of amino acids have been identified that are capable of interacting with CO2 and forming carboxamides that induce excitotoxic neuronal injury, e.g., 

\[ \text{N- \text{methylamino-L-lactic acid (BMAA) and D-aminophosphonic acid (DAP).} \]

Although the parent amino acids bear little resemblance to glutamate, the resulting carboxamides may closely resemble the diaminonic amino acid charge shared by most EAA agonists. In the present study a number of these potentially neuroactive amino acids were tested to determine if their resulting carboxamides could also inhibit high-affinity, sodium-dependent uptake from intravital renal slices electrically, in the presence and absence of NaClO4 (20 mM), as blockers of the uptake of H^+-D-aspartate into rat forebrain synaptosomes. Compounds found to be inactive as inhibitors, either by themselves or which did not include D- and L-carbamates, are listed in Table 1. The uptake did not change in the presence of 300 M NaClO4. Further experiments are under way to determine the specificity of this inhibition.

730.8 CALCIUM-INDEPENDENT EXTRACELLULAR GLUTAMATE SHIFTS EVOKED BY GLUTAMATE. S.E. Smith and M. Cheshier Dept. of Physiology, Neurosurg., NYU Med. Ctr., 550 1st Ave., NY, 10016.

Extracellular alkaline shifts evoked by glutamate (GLU) receptors are generated by a Ca^2+--dependent mechanism [1, 2]. We tested whether other alkalinating processes are unveiled with zero Ca^2+ (EGTA) media. Alkaline shifts were evoked in hippocampal slices (CA1) by pressure ejection of agonists near a pH microelectrode. In zero Ca^2+, GLU, AMPA and NMDA caused no alkaline shift, or a small acidification. Slowing buffer kinetics with the carbonic anhydrase inhibitor benzolamide (10 M) uncovered a slow alkalization evoked by GLU (0.03 ± 0.01 pH units, n=5 slices) but not by AMPA, NMDA or the metabotropic agonist trans-ACPD. A similar Ca^2+--independent alkaline shift was not followed by a GLU-evoked alkaline trans-2,4-DPC (0.05 ± 0.01 pH units n=6), a GLU uptake inhibitor transported by the GLU carrier. Both GLU and L-trans-2,4-DPC-evoked alkaline shifts persisted in the absence of APV (50 M) and CNQX (≤ 100 M). These observations are consistent with efflux of base by the GLU transporter [3]. Since detection of the Ca^2+--independent mechanism required greatly diminished buffering, its contribution to bulk extracellular alkaline shifts is normally minimal. Supported by NIH grant NS32123. [1] Paasmaa et al. (1994) J. Neurophysiol. 72(4):2031. [2] Smith et al. (1994) NeuroReport 5:2441. [3] Bourvill et al. (1992) Nature 360:471.

730.9 EXPRESSION OF NEURONAL AND GLIAL GLUTAMATE TRANSPORTERS IN THE RAT OPTIC NERVE. L. Chiu, S.Y. Chu, and J. Rothman. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706, Johns Hopkins Univ., Dept. of Neurology, Baltimore, MD 21217.

The rat optic nerve consists only of axons and glia, and are devoid of classical synapses. Recent studies, however, revealed dynamic axon-glial signaling in this nerve postulated to be mediated by non-vascular release of glutamate (Krieger & Chiu, 1993). One release mechanism for glutamate in the optic nerve is reversal of high-affinity glutamatergic neurotransmission and the expression of two glial glutamate transporters (GLAST and GLT) and a neuronal transporter (EAAC1) in the rat optic nerve. RT-PCR analysis revealed the presence of mRNA for GLT and GLAST, but not EAAC1, in rat optic nerves. In vitro, in the presence of 6 mM Co^2+, the activity of the sodium-dependent co-transport system was used to quantify the mRNA expression using a housekeeping gene, GAPDH, to control for RNA loading. Of the two glial transporters, mRNA for GLAST is expressed at about 20 times higher than GLT, both in developing and in adult rat optic nerves. GLAST mRNA level in the optic nerve is about 2-fold higher than in cortex (grey matter). Intriguingly, optic nerve expresses slightly higher level of GLAST mRNA than cerebellum, a brain region previously shown to express the highest level of GLAST. Developmentally, GLAST mRNA level in the rat optic nerve is highest at P2 and drops slightly by 6 days post-transplantation. Western blot analysis performed on rat optic using specific antibody raised against GLT, GLAST and EAAC1 revealed major bands of 72, 70, and 70 KDa respectively. Light-level immunocytochemistry shows that GLT and GLAST stain glial processes, EAAC1 stains axons. In conclusion, we suggest that these glial and neuronal transporters mediate neurotransmitter signaling in the optic nerve, by modulating glutamate level in the perisomatic space during nerve activity.

In particular, the unusually high level of expression of GLAST in the optic nerve suggests novel roles for this glial transporter in the optic nerve.

730.10 EFFECTS OF NATRIURETIC PEPTIDES ON SODIUM TRANSPORT AND GLUTAMATE UPTAKE IN RAT BRAIN ASTROCYTES. C.F. Deschepper, S. Picard, K.I. Grove, E.L. Stoehr, and R. Couy. IRCM, Montreal, Canada H2W 1R7.

Natriuretic peptides (NP) in the brain have been shown to control a variety of neuronal functions. The actions of these peptides are generally believed to be mediated via the intracellular generation of cGMP. Since astrocytes appear to be the only brain cells generating cGMP in response to NP, we investigated the effects of C-type NP (CNP) and C-type NP on secondary cultures of rat brain type 1 astrocytes. We observed that CNP 10^4 M decreased intracellular free sodium (as determined by fluorescence of SBFI) in astrocytes. Conversely, intracellular pH (as determined by fluorescence of BCECF) was decreased by CNP, in a dose-dependent fashion. By exposure to CNP. Both effects were mimicked by membrane-permeant analogs of cGMP, and were most likely to be carried out by the GuH+-antipporter, since they were blocked by pre-incubation of the cells with the specific inhibitor 5-(N-ethylcarboxamidomethyl)-alanine (mRNA). In contrast, CNP and other cGMP analogs had no effect on a K+-ATPase function nor on NaK2Cl transport. We also found that both CNP 10^4 M and 8-bromo cGMP 10^4 M diminished the uptake of [3H]-glutamate by lowering the V_m but without affecting the K_m values. This transport could be enhanced by inhibition of the NaH+-antipporter, since H+ had a similar effect. However, glutamate uptake could be decreased even further by CNP in H+-preincubated cells, indicating that this peptide could affect glutamate uptake by an additional mechanism independent of Na+-H+ exchange. Activation of protein kinase G (PKG) is the most likely mechanism, since the effect of CNP was blocked by KT5823, a specific PKG inhibitor. The effects of CNP on NaH+-exchange and glutamate uptake in astrocytes may constitute a mechanism by which these cells could enhance the actions of peptide on neighboring neurons. (Supported by MRC and HSF Canada).

730.11 EPSC SHAPING, AND GLUTAMATE RELEASE IN ISCHAEMIA, BY GLUTAMATE UPTAKE CARRIERS. Michiaki Takahashi, Brian Billette, Mongeau Sarantis and David Altmann. Dept. of Physiology, University College London, Gower St., London, WC1E 6BT, England.

Glutamate uptake is often thought of as being preynaptic or in glial cells, but accumulating evidence suggests that rat cerebellar Purkinje cells express EAAC1 carriers (Neuron 13, 713). By applying glutamate analogues iontophoretically to Purkinje cells in cerebellar slices, we detected a membrane transporter component attributable to glutamate uptake. To investigate the role of this postsynaptic uptake, currents evoked by climbing fibre stimulation were recorded. By altering intracellular ions via the patch pipette, we observed changes of the EPSCs in the presence and absence of glutamate uptake. Inhibiting uptake by removing Ca^2+ (countertransported by the uptake carrier) or adding D-aspartate to the pipette slowed the EPSC decay. These data suggest that removal of glutamate by the synaptic clcptic by postsynaptic uptake can shape the EPSC. In brain ischaemia [KCl]_o rises, making the uptake carrier reverse. We measured the current change by removing glutamate uptake by GABA. In excitotoxicity, we observed that the amplitude of the EPSC was reduced, the decay slowed, and the release of glutamate produced by glutamate depolarisation in raised [KCl]. These data suggest that the acidification occurring in ischaemia may be attributable to glutamate uptake by reversed uptake, and thus protect neurons against transient (but not sustained) ischaemia.

Supported by the Wellcome Trust and the M.R.C.

The glutamate transporter terminates physiological action of excitatory amino acid glutamate in the synapse. In the present study, in situ hybridizations were employed to clarify developmental regulation of three glutamate transporter subtypes (GLUT1, GLUT1, and EAAC1) in the mouse brain embryonic day 13 to postnatal day 21. The GLUT1 mRNA was mostly expressed in the ventricular (proliferative) zone throughout the brain during embryonic stages. In the late embryonic and postnatal period, the expression in the ventricular zone gradually diminished and disappeared, while that in the mantle zone increased progressively. In contrast, the expression increase was outstanding in the superficial layer of the cerebellum (Bergmann glial cells). The GLUT1 mRNA was also expressed in the ventricular zone, but it was virtually restricted to the telencephalon at perinatal stages. During the second postnatal week, the expression levels were augmented prominently in the telencephalon, including the cerebral cortex, hippocampal CA3, and septum. In contrast, the EAAC1 mRNA in the ventricular zone was below the detection threshold at any developmental stages. After postnatal maturity, signals for the EAAC1 mRNA increased gradually over the brain gray matter, with higher levels in the hippocampal CA1 region and dentate gyrus. These findings suggest that the glutamate transporter could play an important role in early differentiation of the brain, in which the transporter subtypes would be involved differentially.


Using rat retinal preparations, we observed a characteristic glial swelling induced by amino-3-phosphonopropionic acid (A3PDA). A3PDA is a potent inhibitor of the excitatory amino acid transporters (EAATs). AP3 is also known to have properties of mGluRs antagonists. Thus, we examined whether the effects of A3PDA were through the modulation of mGluRs. However, although A3PDA reversed inward currents elicited by compound protein CQ (mGluR agonist) or 1-sulfonylaminocyclopentane-1-carboxylic acid (CIC, mGluR antagonist), the results suggest that the modulation of mGluRs activation, or inhibition, was not likely responsible for the glial swelling. Furthermore, the effects of A3PDA was not prevented by CQ or memantine. These results suggest that A3PDA inhibits glutamate uptake. These results suggest that A3PDA induces glial swelling as the retina by serving as a substrate for glutamate transporters. Taken together, A3PDA may be a useful substrate to elucidate the mechanism of glial swelling, which is also produced by glutamate but is not prevented by CQ, MK-801 or mGluR antagonist.
730.19
MOLLEcular Knockout of NeURONAL but NOT GlutAMate TRANSPORT PRODUCES EPILEPSY. M. Balents-Hoberg, J. Bahrami, Y. Wang, D. Schlichter, D. Wehby, and D. Robinson. Johns Hopkins Univ. Dept. of Neurology, Baltimore, MD 21224; and Parke Davis, Ann Arbor, MI.

Glutamate transporter is believed to be essential for the inactivation of synaptically released glutamate. Three glutamate transporters have been cloned: EAAC1 is specific for neurons, whileGLT-1 and GLAST have an astroglial localization. Both EAAC1 andGLT-1 have the highest brain expression in the hippocampus. To understand the role of each transporter in glutamate neurotransmission, antisense oligonucleotides (ODN) were used to selectively inhibit the synthesis of individual transporter subtypes. Antisense ODN were administered intraventricularly over a 7-day period by mini-osmotic pumps (1μl/hr, 10mM ODN/day). Hippocampal EAAC1, GLT-1, and GLAST protein levels were each decreased by more than 50% after one week of individual chronic antiseNSE treatment as measured by immunoblot analysis. Functional glutamate transport was decreased in hippocampus by about 20% after EAAC1 antisense ODN, 50% after GLT-1 antisense ODN, and 20% after GLAST ODN. Furthermore, antisense ODN and random ODN (containing identical proportions of each nucleotide) for each transporter did not alter transporter protein levels, functional transport, or have behavioral effects. Preliminary microdialysis studies indicated that antisense knockdown of glutamate transporter elevated extracellular glutamate levels. Behaviorally, after 7 days treatment, 4 out of 8 EAAC1 antisense treated animals developed hyper-irritability, and stimulus-evoked tonic seizures. By 7 days of treatment, 7 out of 8 EAAC1 antisense treated rats exhibited tonic, and occasionally tonic-clonic seizures. Only 2 out of 11 GLT-1 antisense treated rats developed tonic seizures and none of the 10 GLAST antisense treated rats developed seizures after 7 days of treatment. These studies suggest that dysfunction of neuronal glutamate transport, and to a small extent glial glutamate transport, contributes to glutamate induced seizures.

730.21
CHARACTERIZATION OF THE RELEASE OF DOPAMINE AND 1-METHYL-4-PHENYL-1,2,3,6-PYRIDINUM(DPNP) THROUGH THE RAT DOPAMINE TRANSPORTER EXPRESSED IN COS CELLS. S. Kiyama', K. Morita, and T. Doi. Dept. of Pharmacology, Hiroshima Univ. Sch. of Dentistry, Kasumi 1-2-3, Minami-ku, Hiroshima 734, JAPAN.

In addition to the principal role of neurotransmitter transporter in terminating synaptic transmission by reaccumulating released neurotransmitter, the transport collects the transport process participates in the neurotransmitter release observed in certain physiological condition while distinguishable from vesicular release. To explore physiological relevance of the transporter-mediated release in relation to the action of psychostimulant such as cocaine or neurotoxin, we characterized the release of dopamine(DA) and the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium(MPP+). through the rat DA transporters expressed in COS cells. COS cells expressing the transporter re-activated the ability to release the preloaded [3H]DA and [3H]MPP+ in different degrees and time-course. Release of [3H]DA was enhanced in the presence of extracellular DA, but not affected by cocaine or GBR-12905. On the other hand, release of [3H]MPP+ was enhanced by DA and MPP+ while it was inhibited by cocaine, nomifensine and GBR-12905. These different properties between the transporter-mediated releases of DA and MPP+ suggest the different mode of translocation / reorientation of the transporter for these substrates. This might be important for the re-distribution of substrates for the transporter, especially for the neurotoxic substance such as MPP+.

730.22
GLUTAMATE UPTAKE IS UNCOUPLED FROM THE COUNTERTRANSPORT OF HYDROXYL ion BY VERY LOW CONCENTRATIONS of HgCl2. Z. N. Nagaria and N. Brooks. Dept. of Pharmacol. & Epplt. Therap., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Uptake of l-Glu-GLUT transporters shows closely related exo-intracellular movements (they contain 10 exons interrupted by 9 introns). On the other hand, the gene encoding the neuronal subtype of glutamate transporter is comprised of 12 exons. The cloning and functional analyses of mouse three subtypes of glutamate transporter cDNAs and genes provide a basis for future research into the structure, function, pharmacology and the in vivo functional roles of glutamate transporters.

730.23
DOWN REGULATION OF GLIAL GLUTAMATE TRANSPORTERS FOLLOWING FIMBRIA-FORNIX (FF) TRANSECTIONS AND CORTICOSTRIATAL LESIONS. G. Rothstein, J. Martin, and D. Ginsberg. Dept. of Neurology, and Neurosurgery Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21287.

High-affinity sodium-dependent glutamate transport is the primary means to lower extracellular glutamate levels. Three glutamate transporters have been cloned: EAAC1 is localized to neurons, while GLT-1 and GLAST are astroglial. The expression of glutamate transporter subtypes in rats was evaluated following: (a) unilateral FF transections to destroy the corpus striatum, and bilateral hippocampal ischemia to the septum and hypothalamus, or (b) unilateral lesions of motor-sensory cortex to remove glutamatergic corticothalamic input. The hippocampus and striatum were collected from animals sacrificed at 3, 14, and 30 days and tissue homogenates were assayed for functional glutamate transport and 2) immunoblotted using GLT-1, GLAST, EAAC1, and GP-6, a marker for astrocytes. GLT-1 and GLAST immunoreactivity was significantly decreased in hippocampal ipsilateral to the FF transections at 7 and 14 days postinjection, and total hippocampal glutamate transport was decreased ipsilaterally by 50% at 7 and 14 days postinjection. GLT-1 and GLAST immunoreactivity was significantly decreased within the striatum ipsilateral to the corito-cocellular lesions at 7 and 14 days postinjection, and total striatal glutamate transport was decreased ipsilaterally by 60% at 7 and 14 days postinjection. By 30 days postinjection, glial glutamate transporter protein immunoreactivity and functional glutamate transport returned to control levels. The decrease in astrocytic glutamate transporters was associated with an increase, rather than a decrease, in GPAP immunoreactivity. No significant changes in EAAC1 immunoreactivity were observed at any of the postinjection time points. The study demonstrates that alterations in glial, rather than neuronal, glutamate transporters occur following axotomy of glutamatergic pathways. We hypothesize that glial glutamate transporters may play a dynamic role in plasticity associated with hippocampal and striatal disconnection.
730.27 POSSIBLE REGULATION OF GLUTAMATE UPTAKE BY SECOND MESSENGERS J.G. Ortiz*, O. Claudia, G. Santigato, A. Echeverría, Dept. of Pharmacology, Univ. of Puerto Rico Medical School, PO Box 365067, San Juan, Puerto Rico 00936-5067

Glutamate (Glu) reuptake is the primary mechanism of its removal from the synapse. Several Glu transporters have been cloned. Glycine decarboxylase and its possible involvement in kinase C (PKC) appear as a common feature of the cloned transporters (Kanai et al., 1993). Glu uptake institutionalizes an action that is crucial for synaptic transmission and exhibits a high degree of kinetic characteristics. The process of Glu uptake is modulated by various factors such as neurotransmitters, neuromodulators, and physiological changes. The present study investigated the role of second messengers in regulating Glu uptake.

730.26 ION FLUXES THROUGH EXCITATORY AMINO ACID TRANSPORTERS (J. Wadiche, E.W. McCaman*, S.G. Amara, M.P. Krasneva, Vollum Institute, OHSU, Portland OR 97201)

Currents and uptake of radiolabeled excitatory amino acids mediated by cloned human excitatory amino acid transporters (EAATs) were measured in Xenopus oocytes under voltage clamp in Xenopus oocytes. Consistent with a model predicting translocation of the transporter's subunits, superfusion of the transport substrate D-aspartate induced inward currents in oocytes expressing EAAT2 which did not reverse at potentials up to +80 mV. In contrast, D-aspartate induced currents which reversed in oocytes expressing EAAT1 (Erev = +0.3 ± 0.7 mV; n=46) and EAAT3 (Erev = +38.0 ± 2.7 mV; n=28). The reversal potentials of the EAAT1 and EAAT3 currents shifted 54 ± 1.8 (n=6) and 53.7 ± 4.3 mV (n=10) per 10-fold change in [Ca2+]. The outward currents were abolished upon substitution of extracellular chloride with gluconate. The component of the current carried by chloride was resolved by subtraction of currents recorded before and after depletion of internal chloride and extracellular chloride; this current reversed near ECl. For EAAT1, the quantity of charge translocated per molecule of (H)D-aspartate varied as a function of membrane potential (from +3.5 e- - 100 mV to -2.5 e+ at +25 mV) and was approximately +1 at ECl or when chloride ions were absent. The uptake of (H)D-aspartate was not thermodynamically coupled to the chloride electrochemical gradient. The selectivity sequence of the excitatory amino acid-activated conductance was NO3->Cl->Br->F->gluconate. The results suggest that the net current flowing through the transporter reflects translocation of one net positive charge per transport cycle plus a second current resulting from activation of a gated channel-like activity. This novel behavior provides a potential mechanism for the transporters to dampen cell excitability in addition to removal of transmitter.

SECOND MESSENGERS: G-PROTEINS

731.2 SIGNALING PATHWAYS OF THE DOPAMINE D2 RECEPTOR: DISSECTION USING PERTUSSIS TOXIN-INSENSITIVE G PROTEIN MUTANTS M.H.Griebenzohn* and P. Alberts, Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G 1Y6.

The dopamine D2 receptor coupling to multiple signalling pathways through pertussis toxin (PTX) sensitive heterotrimeric guanine nucleotide binding proteins (Gα). When transfected in Ltk- fibroblast cells (L2D2), the dopamine D2 receptor decreases forskolin-stimulated levels and increases PI inositol phosphate levels. Phospholipase C, and CA2+ and calcium mobilization (Liu et al., 1992), actions which were blocked by PTX pretreatment. In order to investigate the different subtypes of Gα, that mediate D2 signalling, mutation of the Gαi/o proteins which render insensitive to PTX treatment were made. The Gαi/o proteins in the t subunit of Gαi/o proteins (αi/o, αi/o, and αi/o) was converted to by site-directed mutagenesis. Gαi/o proteins were separately transfected in L2D2 cells and cloned were detected by Northern analysis. The amount of protein expressed in each clones was determined by Western Blot using antibodies against each protein. Gαi/o proteins found to be the dominant Gαi/o protein expressed endogenously at both mRNA and protein levels; Gαi/o was at the level of detection. Clones transfected stably with mutant Gαi/o proteins showed levels of species which varied from one to several-fold lower levels of Gαi/o. To study the role of these Gαi/o proteins in signaling of the D2 receptor, [35]GTPαs was measured using Fura-2 in clones expressing mutant Gαi/o proteins. Clones that express mutant Gαi/o protein comparable to endogenous levels were treated with PTX (50 ng/ml, 16h). This elimination of the function of endogenous PTX in activation is observed, while the mutant Gαi/o protein remains unaltered. Preliminary results indicate that the D2 receptor couples most strongly to Gαi/o or Gβ/i γ3 to enhance calcium mobilization. The role of G protein subtypes in receptor coupling to adenyl cyclase and PI turnover is being examined. The mutant Gαi/o proteins provide a useful tool for the investigation of G protein coupling to receptors.
731.3  
ANTISENSE KNOCKOUT OF THE STIMULATORY-TYPE G-Protein, GOLF, IN THE RAT BASAL GANGLIA

Stimulatory-type guanine nucleotide binding proteins (G-proteins) couple neurotransmitter- and hormone-activated receptors to the stimulation of adenylyl cyclase. This study determined the role of Golf, the G protein that catalyzes the formation of the intracellular second messenger, cyclic adenosine monophosphate (cAMP). We have previously shown that Golf-oligofac (Golf) α protein and its mRNA are selectively localized to the neural tissue of the caudate-putamen, nucleus accumbens, and olfactory tubercle. Striatal and striato-pallidal nerve terminals appear also to contain Golf protein. This distribution suggests that Golf may couple the D1 dopamine and or D2a adenosine receptors found in these brain regions to adenyl cyclase. To investigate this possibility, we have used intrastriatal antisense injections with oligonucleotides complementary to bases surrounding the initiation region of Golf mRNA, while oligonucleotides complementary to this region in Gex mRNA have been used as control. Oligonucleotides are 17-20 bases in length with a phosphorothioate modified backbone. Immunohistochemistry of the striatum at 24 and 48 hours post-injection show block of Golf protein expression by Golf antisense oligonucleotides, while Western blots confirm the loss of a 43-44 KD protein, consistent with the loss of Golf. These antisense oligonucleotides will be used to investigate the functional role of Golf in the brain.

731.5  
CORTICOSTERONE ALTERS G PROTEINS IN THE HIPPOCAMPUS.
D.Y. Ohihara*, S.G. Beck and N.A. Mima. Dept. of Pharmacology, Loyola University Medical Center, Maywood, Ill. 60153.

The highest concentration of glucocorticoid receptors is located in the hippocampus, an area of the brain associated with memory and emotions. Several laboratories have demonstrated that the glucocorticoid corticosterone (CT) alters the response of several neurotransmitter receptors in the hippocampus that are linked to several types of G-proteins. We used Western blot analysis and immunohistochemistry to determine the effects of CT on G protein α-subunit (G protein, α, Gα2 and Gα3) levels and distribution in the hippocampus. Four treatment groups were used in our investigation: SHAM, ADX (bilateral adrenalectomy), BX (ADX+12.5 mg CT pellet to produce basal plasma CT levels), HCT (ADX+300 mg CT pellet to produce stress levels of CT in the plasma within 4 weeks). In Western blots ADX had no effect on G protein levels when compared to SHAM animals. BS increased Gα3 levels while HCT increased the levels of Gα1, Gα2, and Gα3 when compared to SHAM. In immunohistochemistry experiments BS increased Gα2 levels in subfield CA3 when compared to ADX. BS increased Gα2 levels in CA1 when compared to SHAM. BS also increased Gα2 levels in the dentate when compared to ADX, SHAM and CT. We conclude that 1) CT does not alter the intracellular distribution of G proteins in the hippocampus, 2) CT alters the G protein levels in the hippocampus and 3) changes in G protein levels may be a compensatory response. Supported by MH-00880 and NS-28512 to S.G.B. and BRSG784 to N.A.M. and S.G.B.

731.7  
MORPHINE- (μ), NALBZOH-(α), AND MORPHINE-β-GLUCRONIDE- (MβG) MEDIATED ANALGESIC SYSTEMS ARE DIFFERENTIALLY BLOCKED BY ANTISENSE DNA DIRECTED AGAINST DISTINCT G-PROTEIN α SUBUNITS. Standifer, R.M.* Ross, G.C.* and Paterson, G.W.* Cotzas Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Opioids mediate analgesia through direct spinal and supraspinal pathways, each pathway containing at least one μ, δ, and κ receptor subtype. Opioid receptor activity is permissive sensitive, indicating G-protein α subunit involvement. Recently, several groups have tried to determine which G protein α subunits responsible for mediating opioid analgesia. Administration of antisense DNA directed against Gαi, Gαo, or Gαs, reduced supraspinal analgesia in mice tested 18-24 hr later (Raffa et al., 1994, Eur. J. Pharmac., 258, R3). We now present data indicating μ, δ, and κ and MβG-mediated analgesic systems show distinct sensitivity profiles with respect to antisense DNA targeted against various G-protein α subunits. Mice (male, CD-1, 25-30 g) were injected with antisense to Gαi, Gαo, Gαs, or Gαq (5μg, i.c.v.). The effects of this antisense DNA treatment on morphine (700 ng, i.c.v.), MβG (25 μg, i.c.v. or NaBzOH (15 μg, i.c.v.) mediated analgesia was assessed using the radiant heat tailflick assay. NaBzOH-induced analgesia was completely blocked 24 hours after administration of Gαo, Gαs, or Gαq antisense DNA (p<0.01), but was unaffected by Gαi antisense DNA treatment. Morphine- and MβG-mediated analgesia were reduced only by administration of antisense DNA directed against Gαq (p=0.01) or Gαo (p<0.05), respectively. These results are consistent with the existence of distinct μ, δ, and κ mediated analgesic systems (as well as distinct morphine- and MβG-induced antinociceptive systems), and underscores the complexity of opioid-mediated antinociception.

731.8  
EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS ON THE LEVELS OF VARIOUS SUBTYPES OF G-PROTEINS (Gαs, Gαq, Gαo) IN RAT BRAIN. Y. Dwivedi, S.G. Pandey, and G.N. Pandey. Dept. of Psychiatry, University of Illinois at Chicago, Ill. 60612.

Antidepressants have been shown to cause changes in neurotransmitter receptors and second messenger systems. Several recent studies have coupled effector proteins through G proteins. In order to examine the role of G proteins in the mechanism of action of antidepressants and anti-anxiety drugs, we studied the effect of these drugs on the levels of Gαs, Gαq, and Gαo subunits in rat cortex and hippocampus. Male Sprague-Dawley rats were given i.p. injections of desipramine (10 mg/kg), lithium chloride (2 meq/kg, twice per day), phenelzine (10 mg/kg), buspirone (10 mg/kg) or mCPP (10 mg/kg) daily for 15 days. The rats were sacrificed 24 hrs after the last injection. The levels of expressed Gαs, Gαq, and Gαo were quantified by Western blotting using specific antibodies to cortices and hippocampi of rats treated with different drugs. We observed that lithium caused a significant decrease in the Gαs subunit (36%) in both cortex and hippocampus, while immunoblotting of Gαq and Gαo remained unchanged. There was no significant change in the levels of Gαq, Gαs and Gαo proteins in cortex or hippocampus after desipramine and phenelzine treatment. We did not observe significant changes in immunoblotting of the G protein subunits after treatment with alprazolam, buspirone, or mCPP. However, the immunoblotting of Gq protein in cortex after buspirone treatment tended to decrease and Gq protein in cortex of desipramine treated rats tended to increase, although it did not reach a statistically significant level. These results suggest that Gq protein may be related to the mechanism of action of lithium.
731.1 ANTISENSE CONSTRUCTS DIRECTED AGAINST Gq, Gβ, REDUCE MUSCARINIC M-CURRENT INHIBITION AFTER INTRANUCLEAR INFILTRATION

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Our previous studies with injections of antisense against the Cterm-decapitated G-proteins established that Gq and Gβ are involved in the modulation of M-current in muscarinic receptor gene sequences, as more specific tools to define G-protein involvement in muscarinic receptor signaling and potentiation of the M current. Intraneuronal injection of rat cultured superior cervical ganglion neurons was achieved using a variant of our method for injection of antisense. We verified injection by induction of green fluorescent protein gene expression in C127 cells later. In a parallel series of experiments, we established that cells injected in a similar manner with a cytoplasmic gene probe-driven green fluorescent protein (GFP) was acetylated by a reduction in M-current inhibition by oxotremorine-M (10μM) from 64%±6% (sense-injected cells) to 28.8%±4% in antisense-injected neurons. These data encourage us to believe antisense-expressing constructs will be useful tools for defining the functions of proteins involved in signal transduction in primary neurons.

731.11 TUBULIN REGULATES Gqα-MEDIATED PHOSPHOLIPASE Cβ SIGNALING.

J. S. Popova*, S. G. Zhou, J. Garamszegi and M. R. Rasenick

Commonwealth of Pennsylvania studies have revealed that the cytoskeletal protein tubulin, with the hydrophobic-resistant phosphaullin probe [3H]J2AGTP (adenosine 5')G bound, interacts with the G protein Gq and thus, is a potential candidate for regulation of phospholipase Cβ (PLCβ) activity. To study this phenomenon further, we infected S9 cells with recombinant baculoviruses encoding Gqα and PLCβ, and compared the effects of GppNpH and tubulin-GppNpH (concentration-response) on membrane-associated PLCβ activity using exogenous [3H]J2IP as a substrate. GppNpH stimulated the high basal PLCβ activity in a concentration-dependent manner. Under the same conditions, the higher concentration of tubulin-GppNpH (500nM to 5 μM) was selected to inhibit the enzyme. In order to study the possibility that tubulin has an effect on receptor-dependent stimulation of PLCβ, we infected C6 cells, simultaneously, with baculoviruses carrying M1 muscarinic receptor (E. Ross, Dallas), Gqα or PLCβ|DNAs. The coexpression of these different DNAs was verified by Western Blotting. Receptor binding studies using [3H]J2IP as a ligand revealed a 240 fmol/mg membrane protein of expressed M1 muscarinic receptors with an apparent Kd of 0.16±2 μM. Carbachol (1 μM) increased the transfer of [3H]2AGTP from tubulin (1 μM) to membrane Gqα. Carbachol-stimulated PLCβ activity was increased in a concentration-dependent and saturable manner by GppNpH. Furthermore, carbachol (1 μM) appeared to blunt the inhibition of PLCβ by tubulin-GppNpH. Since calcium is known to increase microtubule depolymerization, these studies suggest a potential role for tubulin in the regulation of phospholipidasecalcium signaling within the cell.

731.12 IDENTIFICATION OF THE G PROTEIN DOMAIN RESPONSIBLE FOR TUBULIN-ACTIVATED SIGNAL TRANSDUCTION.

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Evidence from our laboratory suggests that the G protein tubulin modifies neurotransmitter receptor signaling, activating certain G proteins (Gq, G11 and Gβ1) by transferring GTP to them. In order to elucidate the physiological role of tubulin-Gα interaction, it was necessary to delineate the pharmacological targets involved in this process. Initial protein-protein and chemical-protein studies suggested a common sequence for tubulin binding (NRRWGW) exists in Gqα (2722-2725) and Gβ1 (2756-2759). Tranducin lacks the above sequence and it does not interact with tubulin. To confirm the above data and to determine the relevance of the domain for functional interaction with tubulin, a number of truncated-Gqα chimeras were made, expressed in E. coli and purified. Reconstituted enzyme sites were introduced with PCR in the pG11 expression vector. Under these conditions, either Gα or Gβ protein was expressed in these chimeras. These chimeras were tested for their ability to bind to tubulin and tubulin overlay studies. Functional interaction of the chimeras with tubulin was studied by measuring the transfer of AATG from tubulin to chimeras and stabilization of bond nucleotides. In one chimera, a small region of Gqα (220-229) in transducin sequence was sufficient to allow activation of the molecule by a tubulin. In another chimera, the region between Gβ and Gβ2 was replaced by transducin, activation by tubulin did not occur. Consensus with proteolytic activity, the first chimeric bound tubulin with similar affinity as Gq11 and Gβ1. The second chimera did not bind tubulin. A synthetic peptide corresponding to the suspected tubulin-binding region of Gqα blocked the tubulin-AATG activation of Gq11. The result suggests that the region NNKkW in Gqα is important for binding and activation by tubulin. It is hoped that this synthetic peptide which blocks tubulin activation of Gqα, will be valuable for future studies aimed at determining the "in vivo" significance of the tubulin activation of G proteins.

SECOND MESSAGES IV

732.1 EFFECTS OF INTERLEUKIN-1β AND TUMOR NECROSIS FACTOR α ON PHOSPHOPLID METABOLITES IN C6 GLIOMA CELLS.

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Cytokines have been implicated in the regulation of astrocytic response to injury. Also, the cytokine inactivation or signal transduction cascades for cytokines in a number of mammalian cells types. The main goal of this work is to examine the involvement of phospholipase activation in signal transduction pathways of the cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) using the C6 rat astrocytoma cell line as an in vitro model. IL-1β exposure initiates a biphasic increase in diacylglycerol levels, as measured by high-performance liquid chromatography. This suggests the activation of more than one phospholipase. TNFα-activated diacylglycerol production is also biphasic but continues for a longer time course than that for IL-1β. Both cytokines stimulate diacylglycerol production in a concentration-dependent manner. Phospholipase D hydrolyses phosphatidylcholine into phosphatic acid and choline. Phosphatidic acid can be rapidly dephosphorylated to contribute to diacylglycerol levels. Both IL-1β and TNFα stimulate, in the presence of ethanol, phosphatidyl ethanol production, as measured by [3H]J2AGTP. The increase in diacylglycerol levels suggests that phospholipase D involvement in the presence of ethanol, phosphatidyl ethanol catalyzes the transphosphatidylolysis reaction, producing phosphatidyl ethanol. The involvement of phospholipase D and the extended time course of diacylglycerol production suggest an interaction with protein kinase C. Supported by DOD grant DOD93693A-2 to Tulane/Xavier CBR.
37.3.2  GLUTAMATE RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER IN BERGAMM GLIA CELLS IN CULTURE.  
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Lopano, 3 A. Pinto, 3 and  
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Primary cultures of fetal Bergmann glia cells were prepared from 
14-day-old chick embryos. We identified and characterized glutamate 
metabotropic receptors coupled to phosphoinositide turnover in these 
glia. Glutamate and NMDA induced an 85% increase in [3H]-ino-
sitol phosphates (InsP3) accumulation within 30 minutes in cells 
preincubated with [3H]-myo-inositol in the presence of 10 mM titrionate. 
Glutamate agonists and InsP3 accumulated in stimulated InsP3 accumulation in the 
following order: L-aspartate > glutamate > quisqualate > N-methyl-D-
aspartate > Kainic = Aminoacyclopestenone-L, 13-diacraylate. NMDA-induced 
response was significant since one minute of stimulation. The potency of 
excitatory amino acid analogues for stimulating InsP3 formation was 
determined within a concentration range from 10 nM to 1 mM. The L-
glutamate-induced response was blocked most effectively by MK-801 (100  
M), D-CP934,635 (200 μM), and AP5 and MCPP showed no effect. 
The effect of NMDA was inhibited by verapamil (10 μM), which blocks T-type 
Ca2+ channels in the modulation of synaptic transmission in the 
cerebellum, through their close apposition to glutamatergic synapses in this 
organ. Supported by Grant 3375-M from CONACYT.  

37.3.3  A NOVEL DIACYLGLYCEROL (DG) KINASE ISOZYME (TYPE IV) FROM RAT BRAIN RESEMBLES EYE-SPECIFIC DG KINASE OF DROSOPHILA WHICH IS ENCODED BY RETINAL DEGENERATION GENE.  
J. Kotelevtsev, 1 and H. Kondo. 2 Department of Anatomy Tohoku  
University School of Medicine, Sendai 980, Japan.  
In the process of signal transduction, (DG) kinase is thought 
to be involved in the resynthesis of phosphatidylinositol by 
converting the second messenger inositol 1,4,5-trisphosphate. DG 
kinese is thus regarded as an attenuator of the activity of 
protein kinase C. Recent studies have shown that DG kinase 
can participate in signal transduction through notably G protein-

coupled receptors but also receptor tyrosine kinase. In order to 
clarify the functional significance of DG kinase in various 
cascades, we have so far cloned the DG kinase from rat 
brain cDNA library and characterized their enzymatic 
properties and localizations. In this study we isolated a novel 
cDNA for DG kinase (DGK-IV) from a rat brain cDNA library. This 
cDNA encoded a protein of 929 amino acids with a calculated 
molecular weight of 104kDa. The primary structure of type IV 
was distinct from those of type I, II and III in that IV contained 
no EF-hand motifs and four ankyrin-like repeats were 
attatched to the carboxy terminals. This structural feature of DG 
kinase-IV closely resembles the recently cloned eye-specific DG 
kinese of Drosophila which is encoded by retinal degeneration A gene. 
However, the rat mRNA for type IV was much more 
dominantly detected in the brain than the eye, the expression 
was localized in all neurons, rather intensively in the cerebral 
and cerebellar cortices. These morphological and structural features of 
DG kinase-IV suggest that DG kinase-IV belongs to a new 
family of DG kinase distinct from type I, II and III.  

37.3.5  THE EFFECT OF ETHER LIPIDS ON PHOSPHATIDYLINOSITOL (PI) METABOLISM IN PRIMARY NEURONAL CULTURES OF COTHE.  
S. L. Hammell, 1 P. L. Robinson, 2 and H. S. Young. 1  
Div. Neuropsych, University of Washington, Seattle, WA 98195, USA.  
These studies were developed to examine the involvement of PI 
metabolism in the regulation of neuronal function and behavior. 
We have previously shown that treatments which inhibit PI 
metabolism in primary neuronal cultures result in a diminution 
of neurotransmitter release. We have also shown that the inhibition 
of PI metabolism results in a significant decrease in 
neurite outgrowth. Therefore, we have investigated the 
role of PI metabolism in different cultures of primary 
neuronal cultures. Three groups of experiments 
were conducted with a focus on the following 
issues: 1) the effects of PI turnover on 
nEAA exocytosis, and 2) the effects of PI 
turnover on neurite outgrowth. 

37.3.6  ACTION OF CHOLELA TOXIN ON CARCINOBLAST- 
STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN CEREBELLAR 
NEURONS. J. Raz, 1 S. K. Zalis, 1 and D. P. Doherty.  
Cholera toxin (CTX) and pertussis toxin (PTX) are conventional tools used to 
study the role of G-proteins in receptor-effector interactions. Activation of the 
phosphoinositide (PI)-specific phospholipases by transmitter receptors is mediated 
through PTX-sensitive Galpha, proteins but PTX-insensitive Gbeta, proteins. So far, direct 
regulation of PI-specific phospholipases by ADP-ribosylation by CTX has not been observed. 
In primary cultures of cerebellar granule cells, the stimulation of muscarinic receptors 
by carbethol (Carb) results in enhanced PI hydrolysis that was inhibited by about 50% 
when granule cells were treated overnight with CTX (0.5 μM). 
A similar treatment of cerebellar granule cells with CTX caused 
a drastic decrease of [3H] labeling of a 50 kDa protein band as revealed by 
CTX-dependent ADP-ribosylation of granule cells in the presence of 
[3H]NAD followed by SDS-PAGE. The maximal-basal level of 300 
activity was decreased by CTX. The level of CTX-induced 
activity was decreased by 30% with the loss of 45% of the 300 
activity. These results indicate that the short-term (less than 3 h) action of CTX on 
cerebellar granule cells either promotes a fast degradation and/or modification of 
300 , or inhibits its biosynthesis. However, these changes are not responsible for the 

decrease of CTX-induced PI hydrolysis. In contrast, a long-term (overnight) treatment of granule cells with CTX results in the 
up-regulation of muscarinic receptors from PI hydrolysis through mechanisms 
which are not directly related to the ability of CTX to ADP-ribosylate 300.

Decreases in cholinergic activity accompanied by increases in nor- epinephrine (NE) concentration have been described in Alzheimer's patients. To determine how these changes might affect brain biochemistry, our laboratory has utilized phosphoglycerate, which, in peripheral sympathetic fibers, originating from the superior cervical ganglia, is upregulated in the hippocampus following cholinergic destruction via medial septal lesion. Since sympathetic neurons can be characterized by high levels of gangliobillins (GABA receptors), the effects of the cholinergic destruction alone (CD), (MS lesions + Gx) can clearly be separated from the effects on the sympathetic fibers. After the septal lesion, NI and HS were found to be differentially affected, with a slight decrease in the former and a large increase in the latter, suggesting that the effects of cholinergic destruction are specific to the hippocampus.

PHOSPHOINOSITIDE-DEPLETED (FRG-PI) human hippocampal cell cultures were prepared by cell dissociation and cultivation of cells in the presence of 50% FCS and 10% goat serum. Following 24 hours of cultivation, the cells were cultured with or without 200 nM ATP for additional 24 hours. The cells were then harvested, and total RNA was isolated and analyzed by Northern blotting and reverse transcription polymerase chain reaction (RT-PCR). The results suggest that the effects of cholinergic destruction are specific to the hippocampus and that the inhibitory effects of ATP are mediated by a specific receptor.

Furthermore, the effects of ATP on the expression of iCOX-ir and iCOX-ir mRNA were assessed. The results suggest that the effects of ATP on the expression of iCOX-ir and iCOX-ir mRNA are specific to the hippocampus and that the inhibitory effects of ATP are mediated by a specific receptor.


Inflammation is a complex reaction that includes increased production of powerful, parasite mediated molecules, nitric oxide (NO). NO can modulate the activity of cytoplasmic NO synthases (COX), an enzyme that produces a class of endogenous prostaglandins. We have taken advantage of multiple COX types to be of particular interest to CNS function. COX and NO synthase (NOS) enzymes should be regionally co-localized since diffusion distances for products of these enzymes is relatively small. Accordingly, we have begun to characterize the distribution of COX1 and inducible (COX2) cytoplasmic COX enzymes in CNS (Breder et al., 1993). Block studies established the specificity of staining. Within neocortex, COX1 showed a dense localization to granular and infragranular cells; COX2 was densely localized to supragranular cells, and NOSs were sporadically localized to infragranular cells with many fine fibers being stained. Within allocortex, COX1 was densely localized to cells of layers M5 and COX2 to cells of layers 2,3,4,5. In this same area, nNOS-immunoreactive cells were seen in infragranular layers. Fibers positive for nNOS were densely packed in a putative terminal field in layer 2 of the agranular insular cortex and dorsolateral entorhinal cortex, a region where many COX2 cells were seen. Similar regional co-localizations of COX2 isoforms and nNOS were seen in hippocampus and amygdala.

This study shows that COX isoenzymes and NOSs co-localize to similar brain regions of cortex. Thus, products from these enzymes conceivably could interact in the CNS. Furthermore, since co-localization of these enzymes is seen in resting brain, such interactions may be an important component of normal brain function. 1. Breder, et al. J Comp. Neurol., 1993. 2. Breder et al., J Comp. Neurol., 1995.

T32.17 IN VITRO ACTIVATION OF LIMULUS LIMUS AND CARDIAC PROTEIN KINASE C BY PHORBOL ESTERS AND ARACHIDONIC ACID: DEFICIENCY CATHETERIZED LIMULUS PLA-selected PATHWAY IN PAPILLOMA VACCINE INDUCED TUMORS. M. Hashemi, A. Papini, C. Papini, I. Burnham and L. M. Schmidt, GM08037, Department of Pharmacology, College of Medicine, University of Chicago, Chicago, IL 60637.

Protein kinase C (PKC) is a multifunctional serine/ threonine-activating enzyme and plays an important role in numerous cellular functions. Twelve isoenzymes of PKC have been identified in mammalian tissues by molecular cloning. The isoforms are grouped into three subtypes based on co-factor requirements for activation. The activation of conventional PKC's (cPKC) requires calcium, phospholipid/horme (PS) and diacylglycerol (DAG). Novel PKC's (nPKC) require PS and DAG but are calcium independent. Phorbol esters (PE) can substitute for DAG in the activation of both cPKC's and nPKC's. Atypical PKC's (aPKC's) are not activated by calcium, DAG or PE but by PS and membrane lipids. Our laboratory has shown that PE-stimulated PKC activity was present in all mouse brain extracts. We have evaluated PKC activity in brain extracts of normal animals and glioma bearing animals. Our results suggest that the PKC levels may be increased in tumors compared to normal tissue. This study was supported in part by NIH grant R-01-NS06906 and NCI grant GM-36032 (BHMK).

T32.18 LIPID METABOLISM MODIFICATION IN THE HUMAN NEUROBLASTOMA SK-N-BE DIFFERENTIATED WITH HETIONIC ACID. A. Bathor,* L. Papini, C. Papini, I. Burnham and L. M. Schmidt, GM08037, Department of Pharmacology, College of Medicine, University of Chicago, Chicago, IL 60637.

We have studied the effects of retinoic acid (RA) in the human neuroblastoma SK-N-BE, RA, an analogue of vitamin A, has been shown to inhibit cell growth, to change the morphology of neuroblastoma cells. RA is also an activator of protein kinase C and is potentially useful in the control of lipid homeostasis. To investigate the degree of cell differentiation and the inhibition of cell growth by RA we have measured neurite outgrowth and labeled tissue incorporation. We have evaluated if RA could also modify lipid metabolism in SK-N-BE. The following parameters have been measured: fatty acids (FA) composition of cellular lipids, the conversion of labeled linoleic acid (LA) to longer chain fatty acids (FA), incorporation of labeled glycerol (G) in lipid, and the conversion of tritiated glucosamine (Glc) to glucose in glycogen. All the indicated parameters were evaluated in undifferentiated and RA-differentiated SK-N-BE at different time periods. RA was incubated at the concentration 10 μM. Undifferentiated and differentiated cells were incubated with labeled LA (0.5 μCi/dish, 18 hours) or glyceral (1 μCi/dish, 24 hours). The cells were scapped and extracted, FA composition in HPLC evaluated and Glc. Extracted of LA labeled metabolites was injected in HPLC connected with a radiodetector on line. FA of the n-6 series: arachidonic acid and 22:4 increased, also 22:6 and 22:5 of the n-3 series increased. The conversion of LA was enhanced at 24 hours than after 7 day differentiation. Glyceral incorporation into the main lipid classes differed according to the different incubation periods. After treatment with RA neurite outgrowth was markedly enhanced and cell growth inhibited. Our data indicate a certain modification of lipid parameters, the molecular mechanism involved in RA-differentiation will be investigated.

T32.19 LYPOSODIOPHATIC ACID IS A PUTATIVE MESSENGER IN NEURONS. R. Diaz-Arrastia* and F. Flamholtz. Dept. of Neurology, University of Texas Southwestern Medical Center, Dallas, 75235.

Lyposodipathic acid (LPA) is a messenger molecule which has potent effects in a number of cell types, including neurons. Because LPA is relatively water soluble, it can diffuse in the site of production to neighboring cells, and may play a role during synaptic plasticity and in the response of neurons to injury. We have found that (1) mammalian brain contains a PL-A, activity which preferentially hydrolyses LPA (as opposed to PGE2) to form LPA; (2) rodent and primate cerebral cell lysates produce LPA in response to activation of cell-surface receptors; and (3) exogenously added LPA potentiates cell death induced by glutamate in an in vitro model of excitotoxicity. PA-specific PLA2 was identified in membranes prepared from rat brain tissue. The enzyme was solubilized using 1% cholate and 1 M KCl, and enriched several hundred-fold by gel filtration, anion-exchange, and hydroxyapatite chromatography. The enzyme does not require Ca2+ for activity and while selective for phospholipid polar head groups, does not discriminate between phospholipid classes with different acyl chains in the 2-4 position. [3H]-LPA production was measured in NG108-15 neuroblastoma x glioma cells which had been labelled with [3H]-palmitate. Treatment of cells with ATP or thrombin resulted in 3 - 5 fold increase in levels of [3H]-LPA, an effect which was mimicked by phorbol esters. There was a parallel but quantitatively greater production of [3H]-PA in response to activators. Finally, the effect of exogenously added LPA on excitotoxicity was studied in cultures of NTS-N neurones which had been terminally differentiated by exposure to retinoic acid. Addition of 1 μM LPA increased the amount of LPA released into the medium induced by subthreshold doses of glutamate (100 μM). These results suggest that LPA is a messenger in neurons which may play a role in excitotoxicity.

T32.20 ECLOGON HORMONE STIMULATES THE PRODUCTION OF CAMP-PA WITH LITTLE CHANGE IN DAG LEVELS. P.A. Simpson* and D.B. Morton. AB1 Division of Neurobiology and the Department of Biochemistry, University of Arizona, Tucson, AZ 85721.

Eclogon hormone (EH) is a 62 amino acid neurotensin which triggers oystis behavior at the end of each molt in the tobacco hornworm, Manduca sexta. Stimuli of this behavior by EH is mediated by a rise in cAMP in the central nervous system prior to oystis. Previous work in this laboratory indicates that this rise due to activation of a soluble guanylate cyclase (GC). However, through the intermediates of GC and protein kinase C, GC activation is associated with nitric oxide (NO) production, but is blocked by a number of inhibitors of lipid metabolism, such as phenolphosphate C and phenolphosphate A, diacylglycerol lipase, and lipoprotein lipase. This study examines the action of EH on a variety of lipids in the CNS. Previous studies have shown that EH stimulates the production of inositol triphosphate (IP3) in the nervous system. Here we show that EH is toxic, even for low levels of IP3, similar to those levels in the diet/cycloheximide in response to EH. This is, however, an increase in the levels of diacylglycerol-phosphate-phosphate-cAMP (AMP). The response is increased downstream of DAG as part of the phospholipase C (PLC) signaling cascade. This is seen in both time course and EH sensitivity to the increase in cAMP and is blocked by many of the same inhibitors that block the ECM-activated increase in cAMP. Additionally, the presence of EH decreased the amount of phospholipid in response to EH. These phospholipids include phosphatidic acid and phosphatidylinositol as well as the major membrane phospholipids, phosphatidylcholine, phosphatidylinositol, and phosphatidylserine. Preliminary evidence suggests that exposure of the CNS to EH results in a decrease in the incorporation of [3H]-PA in a number of these phospholipid species.

Supported by NIH training grant NS07936 to PJS and an Alfred P. Sloan Research Fellowship and NIH grant NS29740 to D.BM.
DEHYDROEPIANDROSTERONE INCREASES NEUROPEPTIDE-mRNA LEVELS IN THE STRIATUM IN ADULT RATS

1. Dehydrated, dehydroepiandrosterone (DHEA) rats were injected, once a day, with 10 mg/kg of DHEA for 10 days. The 11beta-HSD1 enzyme was then assayed in the striatum and hypothalamus of these rats. The results showed that DHEA increased the activity of 11beta-HSD1 enzyme, which suggests that DHEA has a role in the regulation of glucocorticoid metabolism.

2. To investigate the effects of DHEA on the neuroendocrine system, we measured the concentration of ACTH, endorphins, and prolactin (PRL) in the plasma of these rats. The results showed that DHEA increased the concentration of ACTH and endorphins, but decreased the concentration of PRL.

3. This study suggests that DHEA has a role in the regulation of the neuroendocrine system and may be a potential target for the treatment of neuroendocrine diseases.

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Most vasoactive peptides first synapse within the nucleus tractus solitarii (NTS) where information is then related to other brain regions that regulate autonomic and cardiovascular responses. We have examined the changes in Fos expression in functionally distinct regions of the NTS and in overlapping regions of the ventrolateral medulla (VLM) of rats subjected to hypertensive stimuli (H.S.) and retrograde traced using a specific antibody to the Fos protein. Male rats were either injected with either Rhodamine-labeled beads or Fluoro-Gold unilaterally into the carotid sinus. Two hours later, the animals were killed and the brains were dissected. The retrogradely labeled neurons were then stained for Fos expression with a polyclonal antibody. H.S. were induced by injecting phenylephrine (90 µg/kg) followed by a receptor antagonist (chlorpromazine, 300 µg/kg i.p.).

733.8 NADPH-DIAPHRAGM ACTIVITY AND FOS EXPRESSION IN BRAIN NUCLEI FOLLOWING NITROGLYCERIN ADMINISTRATION. C.L. Bealcr 1, F.G. Andrade 1, C.A. Ramírez 1,1, E.L. Tassorelli 1,2, C.D. Barros 1, and E. Guevara-Guzmán 1. State University of Campinas, Facultad de Ciencias Biológicas, UNICAMP, Campinas, São Paulo, Brazil.

NTG-induced cerebral vasodilation is associated with a decrease in Fos expression in specific brain regions, such as the paraventricular nucleus (PVN), locus coeruleus (LC), and nucleus tractus solitarius (NTS). However, the mechanisms underlying NTG-induced Fos expression changes and the relationship between NTG and cardiovascular actions of the brain remain poorly understood.

In the present study, we investigated the effects of NTG on Fos expression in the brain of rats treated with various doses of NTG. The rats were anesthetized with sodium pentobarbital and were subjected to intravenous infusion of NTG (100 µg/kg/min) for 30 min. The brains were harvested at different time points after NTG infusion and processed for immunohistochemical analysis. The results showed that NTG strongly increased Fos expression in the NTS, LC, and PVN, but had no effect on Fos expression in the hypothalamus or the amygdala. These findings suggest that NTG-induced Fos expression is regulated by different mechanisms in different brain regions, and that NTG-induced Fos expression is not a general response to hypotensive stimuli.

Pituitaries (neurophysiologically astrogial) from adult rats exhibit morphological changes when intracellar CAMP is raised by activation of adenylate cyclase (Rassmell & Cobbett, Soc. Neurosci., -2096, 1994). Since a component of serum lysophosphatidic acid, reverses β-adrenergic receptor mediated stiellation of rat CS gloma cells (Koscheck & Tes, Exp. Cell Res.: 206,162-166,1993), we have investigated the effects of newborn calf serum (NCS) on CAMP mediated stiellation of cultured pituitaries. The fraction of stiellated pituitaries was significantly increased when cultures were incubated in medium containing forskolin (5μ,M, 0.9±0.1) compared to that seen in control medium (0.06±0.01). The effect of forskolin was significantly reduced when 0.5% NCS was also included in the incubation medium. NCS also blocked stiellation induced by the phosphodiesterase inhibitor IBMX (50μ,M) and by 8bromo-cAMP (10μ,M). These data indicate that serum prevents stiellation of pituitaries by a mechanism independent of intracellar CAMP concentration. Supported by NINDS (NS28206).

733.15 CALCIUM ENTRY FROM EXTRACELLULAR FLUID IS NOT REQUIRED FOR PROLONGED HORMONE SECRETION FROM APESYNA NEUROENDOCRINE CELLS. N.L. Divac, P. De, UCLA School of Medicine, Los Angeles, CA 90024.

In most neurosecretory systems investigated, membrane depolarization opens voltage-gated calcium channels and Ca++ enters the cell from extracellular fluid, stimulating exocytosis of secretory granule contents. This does not seem to be the case for neuroendocrine bag cells of Aplysia that secrete the peptide hormone egg-laying hormone (ELH). Recently we have shown that once a bag-cell electrical afterdischarge is initiated, Ca++ influx is not required for prolonged ELH secretion (Wayne and Frumovitz, 1995). However, it is possible that brief Ca++ entry during the afterdischarge triggers prolonged Ca++ release from intracellular stores thereby stimulating prolonged peptide secretion. In an initial test of this hypothesis, bag-cell preparations maintained in vitro were treated with the calcium ionophore X537A (50μ,M) dissolved in artificial seawater (ASW) containing 5 mM Ca++/710 mM EGTA. This ionophore can carry Ca++ from extracellular and intracellular sources. By blocking entry of Ca++ from extracellular fluid with the calcium chelator, the only source of Ca++ should be from intracellular stores. This treatment stimulated significant ELH release during the entire 120-min treatment period in the absence of action-potential firing. Total ELH release from preparations treated with X537A (1.2±0.6 μg/ml, n=3) was similar to that from a control group treated with normal ASW and in which secretion was stimulated by an electrical afterdischarge (1.5±0.3 μg/ml, n=5). These results show that a sustained rise in intracellular calcium is sufficient to stimulate ELH release in the absence of an electrical afterdischarge, and are consistent with a model of secretion in which peptide hormone release does not require Ca++ influx from extracellular fluid. Supported by NIH NS-33548.

733.17 LONG DISTANCE DIFFUSION OF DEXTRAN ALONG NERVE FIBRE BUNDLES. ASPECTS ON VOLUME TRANSMISSION. B. Riedke1,2, H. Englund1, C. Nicholson3, M. E. Rico3, L. Lindberg1, M. Zolli1,1 F. Arai1,1, P. Kyörälä1,1

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The mapping out of preferential diffusion pathways in the living brain is basic to the concept of Volume Transmission. Texas Red labelled dextran, a marker for the fluid front, was injected into the dorsal side of the brain unilaterally into the neostriatum in rats (0.3-30μg/ml) and evaluated 1 min to 5 h later. Confocal laser microscopy including co-localization analysis in combination with myelin basic protein staining demonstrated that the Texas Red labelled dextran (5 μg, 3 μg/ml) was associated with small channel-like pathways parallel to the myelinated fiber bundles and separate from the MBP-1 immunoreactivity of the fiber bundles. In addition, strong labelling along the myelinated fibre bundles was seen reaching into the entire neostriatum as well as into the corpus callosum and external capsule on the contralateral side. A diffusion in the neuropil was observed with a clearance starting after 30 min. A marked cellular uptake and accumulation of labelled dextran was found in putative perivascular pericytes. Thus, in the living brain preferential extracellular fluid pathways for diffusion may exist along myelinated nerve fiber bundles.

733.14 CHARACTERIZATION AND EXPRESSION OF LOBSTER PREGNATHANES INVOLVED IN METABOLISM, MOLTING AND REPRODUCTION DOMINGUEZ P.V. DE Klein1,2, Susan K.Waddell2, Gerard J.M. Martens2, Ronald Verweij3 and Pauus Van Hert1, 1Department of Animal Physiology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherland. 2Invertebrate Fisheries Section, Biological Station, St Andrews, New Brunswick, Canada. 3EGG 2XO, 3 Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam.

The crustacean hygrolycemic hormones (CHI-A and -B) are primarily involved in the regulation of carbohydrate metabolism while the growth-inhibiting hormone (GIH), inhibits vitellogenesis and belong to a new neuroopeptide family. In order to get more information on the synthesis, storage, release and possible function(s) of CHI-A, CHI-B and GIH during the reproductive cycle, we measured the levels of their mRNA in the X-organ, their peptide storage in the neuroendocrine gland and their hemolymph peptide levels at different stages of the female reproductive cycle. For CHI-A, a high CHI-A mRNA level was found at the previtellogenic stage while CHI-B mRNA levels were higher in the male as well as in the previtellogenic stages when compared to the opposite stages. During previtellogenesis, high storage levels for both CHI-A and CHI-B were found in the sinus gland. In the hemolymph, the total amount of CHI (CHI-A plus -B) was high only during maturation. For GIH, a low level of mRNA in the X-organ and a low amount of the GIH isoform in the sinus gland were found only in the immature stage. In contrast, GIH hemolymph levels were high during the immature and previtellogenic stages. Together, we conclude that CHI-A and -B are probably involved in triggering the onset of vitellogenesis and especially CHI-B seems to be responsible for stimulating ovary maturation before spawning, while GIH prevents the start of vitellogenesis in the ovary. Moreover, the balance between the hemolymph levels of CHI-A and GIH may regulate the synchronization of reproduction and molting during the bi-annual reproductive cycle of the American lobster.

This study used the transynaptic tracer pseudorabies virus (PRV) to determine the location of different neurons and interneurons within CNS pathways that control bladder or penis. Under halothane anaesthesia PRV (10-20µg, 1.7 x 10^6 pfu/ml, Beckat enteric) was stereotaxically injected into the spinal cord of cats. Animals were perfusion-fixed at 60-115 hours later. PRV injected into the bladder, labeled neurons (L-NEU) were identified in the S1-S3 segments of the spinal cord with majority of the L-NEU in S3 and L3. In the sacral cord L-NEU were in the lateral, sacral parasympathetic nucleus (SPN), the dorsal commissure (DC), in the superficial layers of the dorsal horns (DH), and a few L-NEU were in the lumbar region. At the lumbar level L-NEU were in the intermediolateral cell column (IML), in the DC and in the superficial layers of the dorsal horns. As few animals allowed to survive > 96 hours, binucleated labeling was medial to, and overlapping with locus coeruleus (LC) neurons and extending into the periaqueductal gray and the pontine reticular formation. A few L-NEU were also in the cervical and medullary levels of the pons. In two separate animals either the S1-S3 dorsal roots (DRX) or ventral roots (VRX) were transected prior to bladder injection. Following 60 hour survival little labeling was seen the VRX animal. However many labeled parasympathetic neurons (PRNG) and interneurons were seen in the SPN and DC of the DRX animal. After injection of PRV into the penis L-NEU were in the sacral dorsal horn with majority in S3, rostral S2, and L2. In the sacral cord L-NEU were in the DC, the ventral horn and the medullary SPN. L-NEU in the lumbar cord were mainly in the IML. L-NEU were also seen in the brachial medullary to the hemicord to lateral in the LC. This data suggests: (1) that penile PRNG are adjacent to but separate from bladder L-NEU in the sacral cord; (2) there is considerable overlap of the interneurons serving the two organs and (3) that brachial neurons controlling bladder or penis are present in separate regions of the pons. [Supported by NINDS NS01-NS-2-2374 and NS52420 and DK49303].

374.4 MODULATION OF EXTERNAL URETHRAL SPHINCTER (EUS) BY MICROSTIMULATION OF THE SACRAL SPINAL CORD. M.A.Booth*, A.M.Booth*, M.A. Virag, J.P. Cret, and W.C.de Groot. Dept. of Pharmacol., Univ. of Pittsburgh, School of Medicine, Pgh., PA 15261

The purpose of the present study was to determine sites within the sacral spinal cord where electrical stimulation with fine tipped microelectrodes produced EUS contraction or relaxation, either in the presence or absence of accompanying bladder contractions. Both α-chloroisobutyl-noradrenaline and procaterol decongestant unsensitized cats were used in this study. EUS pressure was recorded via a catheter passed transurethrally with its tip located at the level of the EUS base. The base was recorded invasively by a catheter through the bladder dome. Sites which produced EUS contractions were in S1 and caudal S2; of both sensitized and decongestant cats and included D10 and near (200-400µm) lateral to the D12; (3) deep in the ventral horn and ventral funiculus for the cell bodies and ventrolateral to the dorsal horn. Although sites which inhibit EUS were sometimes identified in sensitized animals the EUS relaxations were usually small (10-15% in H2O magnitude) and often difficult to reproduce even in the same animal. EUS relaxation was best demonstrated in decongestant unsensitized cats. Sites which produce EUS relaxation were often preceded by a small EUS contraction. Sites which produced EUS inhibition include: (1) sites in and near the sacral parasympathetic nucleus (SPN), (2) the dorsal horn just dorsolateral and dorsal to the SPN and (3) along the lateral edge of the ventral horn. Evoked bladder activity was not correlated with the magnitude of the EUS relaxation. Some sites (e. in deep in ventral horn and ventral funiculus of S2) produced large bladder contractions with little or no EUS inhibition (often small EUS contractions were seen); while small bladder contractions produced by dorsal horn stimulation were often associated with large EUS relaxations. Some studies suggest that focal microstimulation of the sacral spinal cord may be a useful technique for producing bladder emptying. [Supported by NINDS NS01-NS-2-2374 and NS52420 and DK49303].


Parasympathetic preganglionic neurons (PGN) and interneurons (INT) in the L6-S1 spinal cord are involved in the control of the urinogenital tract and distal bowel. The present study examined the properties of these neurons in the isolated L1-L6 spinal cord (6.1 x 10^5 cells). Extracellular recordings in the SPN region identified two types of neurons. PGN were activated antidromically at high frequencies (100 Hz) at latencies of 7.5-20 ms by electrical stimulation of the L5 dorsal roots (S5). Some neurons were antidromically activated by stimulation of the L5 dorsal roots (S5). The synaptic responses consisted of single spikes (latency of 5 ms) at frequency of at least 1 Hz of ES. INT did not respond to VRT ES but did fire synaptically at 10-25 ms latency to DRT ES. INT exhibited multiple spikes (3-8) in response to a single spike train ES up to 5 Hz. ES duration stimulus of 2s (4-13 ms) latency and late responses (up to 30 ms latency) on L6 and S1 VRT. Neurons labelled by extracellular injection of biocytin in the SPN region were recorded from the L1-L6 segments and were free from no. (10 to 20 to 120 µm). Some neurons have been identified which have long axon-like processes extending deep into the ventral horn which extended into the lateral funiculus and medial to the dorsal column. Small round or oval neurons in the SPN had dendritic oriented processes. Neurons with long lateral dendrites were identified in the region of the dorsal commissure. These neurons might be labeled by retrograde axonal transport of biocytin from the SPN. It is concluded that some PGN and INT in the SPN are afferent and/or efferent neurons of the sacral sympathetic pathways. This suggests that the late component (25-30 ms) of the DRT to VRT responses could reflect in part autonomic neuron firing.
DENURATION AND INFLAMMATION INDUCED INCREASE OF LOW-AFFINITY NGF RECEPTOR IMMUNOREACTIVITY IN THE RAT URINARY BLADDER. Y. Wakahayashi, A. Buchan, and Y. N. Kwok. Dept. of Physiology, University of
British Columbia, Vancouver, B.C. Canada V6T 1Z3
The denatured and inflamed urinary bladder of rats have been shown to be increased following urethral obstruction, denervation or inflammation, thus suggesting that NGF may play a role in the control of bladder function. However, it is not clear whether the distribution and localization of NGF receptors in the urinary bladder. The objective of the present experiments was to examine the immunohistochemical distribution and localization of NGFR in the low-affinity NGF receptor (LNGFR), which binds all neurotrophins, in the male adult rat urinary bladder using a specific antibody (192 lg). In controls LNGFR positive fiber bundles were shown to be present in the muscle layer. In cyclophosphamide (CYP)-induced cystitis animals, LNGFR positive fine fibers were observed in the muscle layer 2 days after CYP treatment (150 mg/kg, i.p.). Three days after unilateral major pelvic ganglionectomy, LNGFR immunoreactivity was also increased in fine fibers of the muscle layer of the denervated side, and this increase peaked at 7 days. Electron microscopic examination showed that reaction products were located on the surface of Schwann cells and on the interface of axons and Schwann cells. Although the significance of the present findings and the role of LNGFR in the urinary bladder are not certain, these receptors may play a role in nerve regeneration, sprouting and/or neural plasticity. (Supported by BCHRF).

Retrograde axonal tracing studies have demonstrated nitric oxide synthase (NOS) positive neural structures in segments of the spinal cord which receive afferent input from the bladder and urethra. Thus, besides being an effector messenger in the lower urinary tract, NOS may be involved in afferent transmission. It is possible that NOS-containing fibres in the denervated sensory fibres, since no clear role of NO in sensory neurons has been established in this tissue. The objective of this study was to investigate if sensory nerves and their immunoreactivity in the suburothelial innervation of the rat lower urinary tract with special reference to NOS-containing fibres. The bladder and urethra from control and capsaicin treated female Sprague Dawley rats were fixed in 4% formaldehyde and the sensory nerves identified using antisera for immunohistochemistry. Calcitonin gene related peptide (CGRP) and substance P (SP)-immunoreactive (IR) nerves were observed in the suburothelial region of the bladder and urethra. NOS-IR fibres were also observed in the suburothelial region, although more space compared to CGRP- and SP-IR fibres. Occasionally, CGRP-, SP- and NOS-IR fibres penetrated into the urothelial still layer. Electron microscopy confirmed the existence of single, unmyelinated and vescicle-containing nerve fibres between the urothelial cells. Capsaicin treatment resulted in disappearance of both CGRP- and SP-IR fibres. In contrast, the number of NOS-IR fibres both in the mucosa and submucosa appeared to be unchanged. In conclusion, the sensitivity to capsaicin and the presence of free nerve endings primarily suggest a sensory role of fibres in the suburothelial/urothelial. Morphological data do not support the existence of NOS in the capsaicin-sensitive afferent nerve population in the rat lower urinary tract.

MUSCARINIC FACILITATION OF TRANSMITTER RELEASE OPERATES VIA PRESYNAPTIC L-TYPE Ca2+ CHANNELS IN THE RAT URINARY BLADDER. G.T. Seemol, M. Yarrett, O. Seregni and W.C. de Groat, Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261.
The effect of N and L type Ca2+ channel blockers were studied on muscarinic receptor mediated facilitation of acetylcholine (ACh) and histamine-induced (H) NE release in the rat urinary bladder strips. After pretreating the strips with choline chloride 2 nM, two periods of electrical stimulation (ES) and electrical stimulation were applied (20 Hz, and 0.25 ms) to increase the release of ACh and NE from nerve terminals. Control strips yielded an S3/S ratio of 0.98±0.06 for NE and 0.9±0.11 for ACh. Drugs were added 20 min before ES. The Ca2+ channel activators, Ra-K+ 8644 (2M) decreased the release of ACh and S3/S of ACh and NE. Application of 3M (NE) 4.4±0.2±0.4±0.2 for NE and 0.2 did not change that of ACh. The L-type channel blocker, nifedipine (1 M) did not change the non-facilitated NE release or ACh release. Transmitter release was facilitated by the cholinase inhibitor, edrophonium (5 M), which produced an S3/S ratio of 6.6±0.3 for ACh and 4.96±1.58 for NE. This facilitation was blocked by atropine (1 M) and not altered by CTX (20 M) (S3/S; ACh 9.02 and NE 3.41). In contrast, 1 M nifedipine significantly reduced the serotonin facilitated release of ACh and NE (S3/S; 2.0±0.5±0.3 and 1.84±0.07, respectively). These results suggest that N-type channels mediate the non-facilitated release of NE, whereas the facilitated release of both ACh and NE is modulated by L-type channels. Previously we have shown that M1 activation facilitates the L-type Ca2+ dependent pathway. It is possible that M1 phosphorylates Ca2+ channels which are an essential link to muscarinic facilitation. Thus, inhibition either of M1 muscarinic receptor or Ca2+ channel activity can prevent the facilitation of NE or ACh release. Supported by the NHS grant DK-56741.

Barrington’s nucleus, a pontine nucleus implicated in micturition, contains numerous CRH neurons that project to the spinal parasympathetic nucleus that innervates the bladder. This study characterized the effects of selective chemical stimulation of the CRH neurons on bladder function and determined whether the role of CRH-Barrington’s projections in these effects. Bladder pressure was continuously recorded during intracranial glutamate microinjection (10 mM, 15-60 nl) in halothane-anesthetized rats. Selective chemical activation of Barrington’s nucleus by glutamate microinjection evoked bladder contractions corresponding to an increase in pressure of 1-5 mm Hg, and this effect was not affected by the CRH antagonist D-Phe-CRH1-24 (3 µg) administered intrathecally (it) increased the magnitude of Barrington’s stimulated contractions. Moreover, the magnitude of this increase was correlated with the magnitude of chemical activation, e.g., bladder contractions evoked by 15, 30 and 60 nl of glutamate were increased by 34, 3% and 100% respectively (n=7). In contrast to D-Phe-CRH1-24, CRH (3 µg, it) decreased Barrington’s stimulated bladder contractions (n=7), and artificial cerebrospinal fluid (3 µl; it; n=5) had no effect. CRH did not alter bladder contractions evoked by it administered N-methyl-D-aspartate (n=8). These results suggest that activation of Barrington’s nucleus releases an excitatory neurotransmitter responsible for bladder contractions, and CRH, which preynaptically inhibits this neurotransmitter. Through this interaction, CRH may regulate the micturition reflex.
734.13
DESENSITIZATION OF BLADDER SENSORY FIBERS WITH INTRAVENOUS CAPSAICIN IN HUMANS WITH DETRUSOR HYPERACTIVITY F. Cruu, M. Guzman, C. Silva, M.E. Rio, M. Reis and A. Coimbra. Institute of Histology and Embryology, Dept. of Urology and Dept. of Neurology, Faculty of Medicine of Porto, 4200-Porto, Portugal

Experimental studies have shown that detrusor hyperactivity very often follows the emergency of a strong spinal micturition reflex triggered by unmyelinated C-fibers. In the treatment of the urinary symptoms associated with this condition desensitization of C-fibers may constitute an alternative to anticholinergic drugs. With this purpose, capsaicin, the pungent extract of red peppers which is known to desensitize cutaneous C-fibers, is currently under investigation. We treated 10 patients with bladder hyperactivity associated with neurological disorders (8 pelvic radioloculation (1) or idiopathic hyperpersistent bladder (1). All patients gave informed written consent. A 1N M capsaicin solution in 30% alcohol was instilled through a urethral catheter and left in contact with the mucosa for 30 minutes. Patients mentioned a burning sensation immediately after capsaicin instillation that began waning slowly 15 minutes later. After 3 months and 8 months treatment, no significant effect was observed. In the meantime the patients had disappeared. The average volume at which first desire to void occurred increased from 103±9ml to 218±11ml (p=0.02) and maximal cystometric capacity increased from 204±51ml to 350±21ml (p<0.001) and 348±17ml (p=0.05) respectively. The patients that completed one year follow-up showed a pronounced deterioration of the clinical condition and the cystometric data returned to pretreatment levels. These findings suggest an important role for bladderafferent desensitization in the treatment of detrusor hyperactivity.

734.14
OPPOSITE EFFECTS OF DULOXETINE, A SEROTONIN (5HT) AND NORADRENALINE (NE) RE-UPTAKE INHIBITOR, ON NOCICEPTIVE REFLEXES TO THE BLADDER AND URETHRAL SPHINCTER. K.B. Thor* and M.A. Katofface. Ei Lilly and Co., Indianapolis, IN 46285

SHT and NE systems are intimately associated with CNS control of LUT function. The present study has examined the effects of duloxetine (DUL) on bladder capacity and urethral sphincter EMG activity in chloralose-anesthetized rats. Cystometric and urethral EMG activity levels were performed under conditions of saline infusion into the bladder or saline followed by dilute acetic acid (0.5%) infusion. Under saline conditions, bladder capacity was significantly reduced (5mL) and urethral EMG activity was low. Duloxetine increased bladder capacity and reduced hyperactivity. By contrast, in the urethral EMG, bladder activity was reduced (hyper-reflexia) and sphincter activity was increased. Duloxetine thus appears to modulate both bladder capacity and sphincter activity during saline infusion. However, during acetic acid infusion, bladder capacity was reduced (hyper-reflexia) and sphincter activity was increased. Duloxetine had weak, nonsignificant effects on bladder capacity and sphincter activity during saline infusion but presented significantly reduced bladder capacity (i.e. increase in capacity) and facilitation of sphincter activity. Various experiments confirmed that the effects were mediated centrally through SHT and/or NE receptors. These results indicate that monoaminergic systems can either inhibit or facilitate nociceptive-driven input depending on the different system studied. Since inhibition of the bladder and facilitation of the sphincter would be a conditioned, appropriate behavior under "fight or flight" conditions, it is tempting to speculate that the monoaminergic system "directs" nociceptive inputs to, or away from, specific efferent systems and may contribute to the physiological changes useful in the context of "fight or flight" situations.

735.1

The sacral parasympathetic nucleus (SPN) contains preganglionic autonomic neurons destined to the pelvic organs, and is the source of prorecticular neurons. Oxytocin (OT) administered into the cerebral ventricles induces penile erection and yawning in the male rat. Oxytocinergic and vasoactive intestinal polypeptide (VIP) axons are present in the caudal part of the hypotalamus. We searched for the presence of oxytocinergic fibers in the SPN using retrograde labeling combined with immunocytochemistry. In adult anesthetized male rats, a solution of wheat-germ agglutinin-horseradish peroxidase (WGA-HRP, 25% in distilled water) was applied on the central cut end of the left pelvic nerve. Rats were sacrificed two days later and serial sections of the lumbosacral spinal cord were treated with tetrodotoxin (a membrane-stabilizing agent) and stained with diaminobenzidine (DAB) complex to reveal retrograde-labeled SPN neurons. Immunocytochemistry was performed on the same sections using an anti-oxytocic antibody stained by the immunoperoxidase method. Retrograde-labeled neurons were localized in the intermediate and lateral columns of the L6 and S1 level of the spinal cord. They formed a dense and homogenous population. At the same levels, OT-negative immunoreactivity was associated with various fibers which were mainly found in the SPN, in the marginal zone and in the dorsal commissure. Rare OT-positive fibers were seen in the ventral horn. In the SPN, OT-immunoreactive fibers appeared in close apposition with retrogradely-labeled neurons. At the ultrastructural level, OT-positive fibers containing DAB precipitates contributed various fibers which surrounded retrogradely-labeled neurons and could make on them synaptic contacts. We conclude that oxytocinergic projections exist on SPN neurons. These results provide a morphological support for supraspinal control of oxytocin-mediated pelvic function.

735.2
THE AUTONOMIC AND SENSORY INNERVATION OF THE RAT PROSTATE. R.E. Mckenna*, C.R. Georges, X.-B. Guan, and K.L. McCary, Dept. of Physiology and Urology, Northwestern University Medical School, Chicago, IL 60611

We have previously demonstrated that the autonomic innervation exerts a trophic influence on the prostate. Specifically, unilateral sympathectomy induced an ipsilateral atrophy, while unilateral parasympathectomy induced a contralateral hypertrophy. The goal of the present study was to examine the sensory innervation of the prostate. Adult male rats were anesthetized and Fluorogold (2 µL, 4%) was injected unilaterally into the ventral prostate under anesthetizing condition. After a 7-10 day survival period, rats were reanesthetized and perfused with fixative. The sympathetic chain, inferior mesenteric and major pelvic ganglia and dorsal root ganglia (DRG) were removed. Labelled neurons were counted in each ganglion. The vast majority of labelled postganglionic neurons were located in the major pelvic ganglia, the mixed sympathetic parasympathetic response ganglion. Some labelled postganglionic neurons were observed in the sympathetic chain. Very few labelled neurons were seen in the inferior mesenteric ganglia. Labelled neurons were observed bilaterally in approximately equal numbers. The afferent cell bodies from the prostate were localized predominantly in the dorsal root ganglia of L6, followed by L5 and S1. Approximately one third of the labelled DRG neurons were located in the T13-L2 segments. Labeling of sensory neurons was observed bilaterally.

735.3
ESTROGEN RECEPTOR-IMMUNOREACTIVE NEURONS ARE PRESENT IN THE FEMALE RAT LUMBOSACRAL SPINAL CORD. J.J. Williams* and R.E. Pappa. Department of Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190

Estrogens are central steroids which stimulate secondary sex characteristics as well as growth, maturation and behavioral patterns in the CNS. Previous studies have examined potential sites of action of estrogen in the CNS by identifying sites of estrogen receptors, e.g., the hypothalamus, preoptic area and the amygdala. However, little attention has been directed to revealing estrogen receptor-containing neurons in the spinal cord, whether such neurons are involved in the innervation of female reproductive organs, or if such neurons project information about reproductive organs to higher centers. A few studies have mentioned, somewhat passing, the presence of estrogen receptors in the spinal cord. Thus, we have undertaken a study of the location and distribution of estrogen receptor-immunoreactive (ER-IR) neurons in the female rat spinal cord by using immunohistochemistry.

Intact and ovariectomized rats were anesthetized, perfused fixed and the spinal cord and brain sampled for immunocytochemistry. Immunoreactive cells were immunostained with a rat monoclonal antibody (H222, Abbott Laboratories). ER-IR were observed mainly in the nuclei of neurons. ER-IR neurons were located predominately in the dorsal one-half of the spinal cord; specific sites containing ER-IR include the dorsal horn, the spinal parasympathetic nucleus, lamina V, lamina X around the central canal and also in the external nucleus. These regions suggest that neurons can be influenced by circulating estrogen. The ER-IR neurons could be involved in transmitting information either peripherally or centrally. (Supported by NIH Grant NS22232 and Presbyterian Health Foundation.)

735.4
EXPRESSION OF ESTROGEN RECEPTOR (ER) IN NEURONS OF RAT SPINAL CORD, DORSAL ROOT GANGLIA (DRG) AND PELVIC AUTONOMIC GANGLIA (PG). B. Brunvand, K.E. Pappas, and K.E. Miller. Dept. Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190

This study was directed to identify spinal cord neurons, sensory neurons and autonomic ganglion neurons that could be responsive to estrogen. Ultimately, we want to use autoradiography or in situ hybridization histochemistry to study estrogen receptor mRNA in central and peripheral neural circuits related to uterine innervation. In situ hybridization histochemistry and immunohistochemistry were used to examine the presence and distribution of neurons exhibiting estrogen receptor (ER) mRNA and ER protein respectively, in the lumbosacral cord levels L6-S1, L6-S1 DRG and PG of ovariectomized (ovx) and intact females. Ovaxed spinal cord sections were prepared for in situ hybridization histochemistry using oligonucleotides [48 bases] labelled with digoxigenin-d-UTP. Immunohistochemistry was performed on sections of aldehyde-fixed spinal cords and ganglia using antisera from several different sources: specific antisera was sectioned and reacted with an ABC reaction. Neurons with ER-IR nuclei were identified in the dorsal one-half of the spinal cord including preganglionic parasympathetic neurons of the greater and lesser splanchnic nerves, and in the dorsal root ganglia. DRG. PG mRNA expression was evident in many neurons in the spinal cord including those areas containing ER-IR neurons. Numerous neurons in the PG and DRG were estrogen receptor positive and were almost exclusively small and medium-size. Labeling was more evident in the ovax rats. These data suggest there are abundant neurons in the spinal cord and peripheral ganglia which could be responsive to circulating estrogen. (Supported in part by NIH grant NS-22526).
IDENTIFICATION OF 5-HT RECEPTOR SUBTYPES INVOLVED IN SEXUAL REFLEXES IN THE SPINAL CORD OF MALE RAT. X-B. Kwon, M. I. Schlegel, J. A. Tashjian, and C. B. McKenzie. Departments of Physiology and Urology, Northwestern University Medical School, Chicago, IL 60611.

We have previously demonstrated that spinal sexual reflexes are under the control of a descending inhibitory 5-HT pathway from the nucleus paragigantoocellularis (nPGi). However, the specific receptor mechanism involved in this process has not been identified. The purpose of this study was to identify the 5-HT receptor subtype(s) involved in the descending control of sexual reflexes. Primers for 5-HT receptor subtypes 5-HT1A, B, C, D, E, F, I, J, K, and 5-HT1A were designed and synthesized. A reverse transcription followed by polymerase chain reaction (RT-PCR) for these three 5-HT receptor subtypes were conducted to observe the expression of these receptors at the message level in the lumbar-sacral segments of the spinal cord. Male Sprague-Dawley rats were used in this study. The spinal cord was dissected into L1-2, L3-4, and L5-S1 segments and then further dissected into dorsal and ventral portions. Total RNA was used for reverse transcription. The PCR products were resolved on a 2% Metaphor agarose gel stained with ethidium bromide.

The expected size of products for all three receptor subtypes were obtained (342 bp for IA, 244 bp for 1B and 494 bp for IF, respectively). Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was co-amplified as an internal control. The density ratio of the 5-HT receptor and G3PDH was used to quantitate the mRNA amount. While the IB receptor has been clearly identified in the lumbar-sacral segments of the spinal cord, the distribution of the other receptor subtypes and the control mechanism are still under investigation.

735.7 CYTOCHROME OXIDASE STAINING IN THE RAT MAJOR PELVIC GANGLIUM (MPG). M. G. Pali-S. Palisao, P. Parli, S. Levyra, and Y. Baha. Dept. of Anatomy, Univ. of New Mexico, Albuquerque, NM 87131.

Pelvic autonomic neurons display a variety of neurochemical and functional differences, which in part reflect the diverse reproductive and eliminative organs they regulate. This study localizes cytochrome oxidase (CO) activity and demonstrates the heterogeneity in pelvic neurons extends to a marker of metabolic activity. A distinct topography is apparent in neurons that stain lightly for CO and are located in the ventral portion of the MPG near the entry of the hypogastric nerve (HN), while darker neurons predominate in the dorsal pole. The most intensely reactive neurons are located in a longitudinal band in the central region of the MPG. Terminals (verified by their disappearance after cutting the HN) around lightly stained neurons vary in size and are foci of intense CO activity, whereas terminals surrounding neurons in the dorsal pole are less apparent. Topographic relationships and immunohistochemistry suggest that large cells with relatively low CO activity are adrenergic neurons while penile neurons (located by retrograde tracer) are moderately active. These findings suggest that the varied basal metabolic activity of neurons in the MPG mirrors the level of synaptic activity needed to regulate respective pelvic organs. Supported by NIH ROI1983-11.

735.8 HORMONAL CONTROL OF CHOLINERGIC, NORADRENERGIC AND PEPTIDERCIC TRANSMITTERS IN AUTONOMIC GANGLIA. R. W. Hamill, J. M. Hayes, and K. A. Elson. Departments of Neurology, and Anatomy & Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

In the spinal sexual reflexes, influences regulate biochemical and morphological features of neurons. Previous studies indicate that androgens influence the neurochemistry of the hypogastric ganglia (HG). Therefore, in adult Sprague- Dawley rats, examine the effects of castration on noradrenergic, cholinergic, and peptides systems in the major pelvic ganglia (MPG), sexually dimorphic ganglia innervating the urinary bladder and reproductive organs in the pelvis. Castration alters the activity of Tyrosine Hydroxylase (TH), the rate limiting enzyme in noradrenaline biosynthesis and a marker of noradrenergic adaptability, and Choline Acetyltransferase (ChAT), a marker of cholinergic components of autonomic ganglia. Four weeks following castration TH and ChAT activities are 25% and 50% of control, respectively. At 8 and 12 weeks post-castration, ChAT levels remain at 50%, whereas TH activity gradually declines reaching a nadir of 15% of control by 12 weeks. Castration also alters the levels of vasoactive intestinal peptide (VIP), which is colocalized with ChAT in MPG. VIP levels are reduced approximately 25% at 4 weeks following surgery. In all paradigms, testosterone replacement therapy fully restores TH, ChAT, and VIP levels. These studies indicate that the biosynthesis of noradrenergic, cholinergic, and peptide neurotransmitters in the adult MPG are regulated by testosterone. Taken together with previous studies of the HG, the results indicate that parasympathetic and sympathetic ganglia involved in reproductive function respond to gonadal steroids during adulthood. (Supported by NS22103-RWH; HD22768 and NS01636-VM; AAH4015540-KMB).


The majority of men with SCI are in the parenting age group. Most of these men suffer from low numbers of motile sperm. This pilot study assessed the efficacy of CC for improving sperm motility in SCI men. Four volunteer SCI men had monthly semen analyses for 6-12 months prior to CC ("CC"), and then again for 6-12 months after CC ("CC+"). CC given as 25 mg orally every other day. The following semen parameters were measured in the "CC" and in the "CC+" conditions: (a) total number of sperm per ejaculate, (b) number of motile sperm per ejaculate, (c) percent motile sperm per ejaculate, (d) total number of sperm with rapid linear motion per ejaculate, (e) percent of motile sperm with rapid linear motion, and (f) the percent motile sperm in the nongrape fraction only. ANOVA was used to evaluate the statistical significance of any changes in the "CC" and "CC+" conditions. All measures of sperm motility improved significantly with CC treatment. There was no difference in the total number of sperm per ejaculate with CC treatment. Based on this small group of subjects, it appears that CC may improve sperm motility in SCI men. To our knowledge, no treatment for SCI men has heretofore been shown to have any efficacy in these subjects. A larger study is needed to evaluate the effect in more SCI men and to evaluate the effect on fecundity.


SCI men have sperm of normal concentration but poor motility (Brackett et al., J Urol. 151:114-119, 1994). No single factor has been identified as a cause for this condition. Objective: The present study investigated the seminal plasma of SCI men contributes to their poor sperm motility. Method: Aliquots of washed sperm from normal men (n=13) were mixed either with seminal plasma from SCI men or with seminal plasma from normal men. Similarly, aliquots of washed sperm from SCI men (n=13) were mixed either with seminal plasma from normal men or with seminal plasma from other SCI men. Results: Percent motile sperm decreased significantly, from 72% to 45% (p<0.01), when mixed with seminal plasma from SCI men. Percent of motile sperm in SCI men was significantly higher when mixed with seminal plasma of normal men (43% vs 34% of normal sperm from SCI men (29%) (p<0.05). Conclusions: Seminal plasma of SCI men appears to contribute to their poor sperm motility. There may be toxic factors present in these recent SCI men who mixed with sperm during ejaculation, in the absence of SCI, to impact sperm motility.
736.1
THE ROLE OF BRAINSTEM RETROTRAPEZOID NUCLEUS (RTN) METABOTROPIC RECEPTORS IN THE PROLONGED STIMULATORY EFFECT OF RESPIRATION PRODUCED BY RTN INJECTION OF GLUTAMATE. Aihua Li and Eugene E. Nam*.
Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756-0001.

Stimulation of metabotropic glutamate receptors (mGlRa) in the RTN of the chloralohorene anesthetized rats by the mGlRa agonist (1S,3R)-
aminocyclopentane-1,2-dicarboxylic acid (AADC) increases integrated phrenic amplitude (PNA) for > 60 min. This long-lasting PNA stimulation can also be mimicked by the long duration (60 sec) injection of glutamate. Here we show the mGlRa agonist (a)-methyl-4-carboxyl-glutamine (c-MCPG) can block both of these long lasting effects. Using multi-barrelled microprobes, we first identified RTN sites associated with respiration by observation of glutamate induced short-lived PNA stimulation. In group 1, five rats received only mGlRa agonist (100 nM); in group 2, rats received mGlRa agonist (100 nM) injection and c-MCPG (100 nM) injection 30 min later. In group 3, five rats received c-MCPG (100 nM) injection and the mGlRa agonist (100 nM) injection 30 min later. In group 4, five rats received c-MCPG injection followed by injection of mGlRa agonist (100 nM) every 10 min until PNA response to glutamate injection was observed. The active antagonist (c-MCPG) had no significant effect on PNA but it blocked a) subsequent IS,3R-ACP induced PNA stimulation for 90±1min and b) the PNA response to glutamate injection for 60±11 min. The inactive form (c-MCPG) had no effect on ACPD-induced PNA stimulation. We conclude: 1) RTN mGlRa are involved in respiratory control, 2) they are not active in awake and 3) their stimulation may require prolonged glutamatergic release. (Supported by HL 28066)

736.2
Dept of Medicine and Centre For Neuroscience, Flinders Medical Centre, Bedford Park, South Australia.

Excitatory respiratory pre-motoneurons are located in the ventral respiratory group (VRG) of the brainstem, while inhibitory pre- 
motoneurons are found in the Bötzingner region. Together they regulate the activity of phrenic motoneurons. In this study we aimed to determine if these brainstem respiratory neurons are immunoreactive for the glutamate synthesizing enzyme, phosphate activated glutaminase (PAG). Respiratory neurons in the ventrolateral medulla of pentobarbitone-anesthetized Sprague-Dawley rats were labelled by intracelular injection of Neobiotin (Vector, CA). After histochemical processing and immunohistochemistry, sections of brainstem were examined by fluorescence and light microscopy. Four types of respiratory neurons were identified. 1) Six inspiratory neurons in the VRG five PAG immunoreactive and one PAG negative. 2) Six expiratory neurons in the VRG: five neurons were PAG immunoreactive and one PAG negative. 3) Five neurons in the Bötzingner area: four PAG negative and one PAG immunoreactive. 4) Seven ambugal motoneurons that were all PAG immunoreactive. Our results support the use of PAG as a marker for glatamatergic respirotory neurons since most of the excitatory VRG neurons contained moderate to strong levels of immunoreactivity while most Bötzingner neurons, which are believed to be inhibitory, contained none. These findings also suggest that glutamate is synthesized and released as a neurotransmitter by excitatory respiratory neurons.

736.3
COMPARISON OF GAD- AND GABA- IMMUNOREACTIVE NERVE TERMINALS IN THE RAT PHRENIC MOTOR NUCLEUS. Susan M. Murphy*, Paul M. Plisvasky and Ida J. Llewellyn-Smith, Dept of Medicine, Flinders Medical Centre, Bedford Park, South Australia, 5042.

y-aminohipuric acid (GABA) mediates synaptic inhibitions of phrenic motoneurons during expiration. About 30% of nerve terminals that make 
synapses or direct contacts with phrenic motoneurons immunoreactive for GAD, the GABA synthetic enzyme, whilst only about 18% of inputs identified by post-embedding immunogold are GABA-positive (SN 1994, Poster 231 B). In this study, we examined the distribution of these two markers in adjacent pairs of ultrathin sections containing retrogradely-labelled phrenic motoneurons. Both sections were labelled for GAD-immunoreactivity by pre-embedding immunocytochemistry, and one section of each pair was subsequently immunogold labelling for GABA. We examined terminals that synapsed on or directly contacted phrenic motoneurons, as well as other non-synaptic terminals. Many terminals containing high densities of gold particles also proved to be GAD-positive. However, not all GAD-immunoreactive nerve terminals were strongly labelled for GABA. Comparisons of the density of gold labelling over GAD-positive and GAD-negative terminals suggest that there is overlap in the frequency distributions of the two populations. Assuming GAD is itself a reliable marker for GABAergic terminals, we must conclude from these and our previous results that in this part of the spinal cord, post-embedding immunocytochemistry for GABA alone does not provide a sufficient basis to discriminate between GABAergic and non-GABAergic terminals that provide synaptic input to phrenic and other motoneurons.

736.4

We previously identified synapses in the lung C-fiber afferent pathway in the medullar commissural nucleus tractus solitarius (cNTS) which were essential for C-fiber evoked a pain and rapid shallow breathing (RSB). We examined the role of EAAs as potential neurotransmitters. Phrenic nerve activity, blood pressure, and heart rate were continuously recorded in urethane-anesthetized, paralyzed, and ventilated rats. C-fibers were stimulated by injecting phenylglyoxal (PGG; 0.05-0.1 ml, 50% glumatic in the right aurum (RA) and left ventricle (LV). In some rats we cut both vagi below the diaphragm and denervated the baroreceptors and chemoreceptors. Medial CNS was screened for vagnaly-evoked units with a 7-barrel electrode containing 2% pontamine dye in 0.5M NaAc for extracellular recording, EAA agonists and antagonists for lophohosphere, and normal saline for balancing currents. RA PGB (n=25) evoked immediate increases in unit activity; (152±18/18Hz) (p=0.004) for 17±20s, that coincided with a pain or RSB. In 9 cells RA PGB compared to LV PGB produced greater increases in activity (14±16 vs 8±10Hz, p < 0.04) for longer durations (85 s vs 3±4 s; p=0.01±0.05). The EAA agonists NMDA (8-30nm; 100mM) and QUIS (8-60nm; 100mM) evoked dose- dependent increases in unit activity. NMDA (24±7/4A) and QUIS (29±15/4A) increased unit activity from 0.5±0.5 to 7±25Hz and from 0.4±5/4 to 11±4Hz, respectively. The NMDA receptor antagonist, AP5 (90 nA, 100 mM) in doses that blocked NMDA-but not QUIS-evoked increases in unit activity, did not alter PGB-evoked increases in activity. Activation of both NMDA and non-NMDA receptors stimulates cNTS neurons in the C-fiber afferent pathway, but NMDA receptors are not involved in neurotransmission. Supported by NIH HL48584.
736.9 MK-801 ALTERS THE RESPIRATORY RESPONSE TO HYPOXIA IN ADULT RATS. S.K. Coles*, M.F. El-Khatib, and T.E. Dick, Dept. of Medicine, Div. of Pulmonary and Critical Care Medicine, Case Western Reserve Univ., Cleveland, OH 44106

Blocking NMDA receptor channels with the non-competitive NMDA antagonist MK-801, profoundly changes pulmonary response to hypoxia in adult rats.

MK-801 (0.5 mg/kg) was administered by intraperitoneal injection followed by hypoxia (5% O2). The respiratory response to hypoxia was monitored by recording tidal volume (TV) and respiratory frequency (f).

Results: MK-801 administration decreased TV and increased f, resulting in a decrease in the respiratory rate (TV/f). These changes were reversible within 30 min after MK-801 administration. MK-801 also altered the response to hypoxia, with a decrease in TV and an increase in f, compared to hypoxia alone.

Conclusion: MK-801 administration alters the respiratory response to hypoxia in adult rats, potentially influencing the response to hypoxia in clinical settings.

Supported by HL-07288 and HL-42040 (TED).

736.10 EFFECTS OF NBQX ON RESPIRATION IN ADULT RATS. Caroline A. Connelly*, Department of Surgery, University of California-Davis, Sacramento, CA 95817


The present study examined the role of non-NMDA receptors in the generation of respiratory rhythm in spontaneously breathing adult rats.

6-Nitro-7-sulphamoylbenzofuran-2,3-dione (NBQX) is an AMPA/kainate receptor antagonist that crosses the blood-brain barrier. NBQX (40 mg/kg, i.p.) was administered to anesthetized Sprague-Dawley rats while diaphragm EMG activity and arterial pressure were monitored. Respiratory frequency significantly decreased (p<0.01, f0.05). Changes in arterial pressure were both prolonged. Significant decreases in arterial pO2 and pH indicated that NBQX depressed ventilation. Arterial pCO2 increased in 5/6 rats. Changes in arterial pressure were insignificant. When the NMDA receptor blocker MK-801 (1 mg/kg, i.v.), was injected 5-15 min after NBQX, respiratory frequency decreased further and arterial pressure was within 45 to 1 at 0.5 min. 5/6 rats had apneic episodes (1.5 to 18 s duration) and/or a significant increase in tidal volume for 15 min prior to the injection of NBQX, indicating that chemosensitive neurons were acutely ventilated until spontaneous breathing resumed after 7-8 min.

These data indicate that non-NMDA receptor activation is significantly involved but not necessary for respiratory rhythm generation in adult rats. These results support the hypothesis that combined activation of NMDA and non-NMDA receptors underlies respiratory transmission necessary for respiratory rhythm generation. NBQX was generously provided by Novo Nordisk.
SEROTONIN IN THE DORSAL RESPIRATORY GROUP DECREASES HYPOGLOSSAL (XII) NEURAL ACTIVITY. M. A. Douceur, E. J. Puglisi and D. P. White, Dept of Medicine, UCHSC, and Respiratory Care, VMAC, Denver, CO 80220.

Serotonin (5-HT) is known to increase XII motoneuron activity when applied directly to the XII nucleus, but nothing is known concerning 5-HT modulation of XII activity via other integration sites in the brainstem. We determined the effects of 5HT and methysergide (broad 5HT antagonist) pressure microinjection into the dorsal respiratory group (DRG; 25-100 nL; pH=7.2-7.4) in XII whole nerve activity, measured as the inspiratory peak height of XII integrated neural activity (time constant=100 msec). XII neural activity ipsilaterally to the injection site significantly decreased to 85±5.4% (25 nL), 74.2±1.5% (50 nL), and 50.4±10.5% (100 nL) of control values (n=5, all p<0.05). Contrastral XII activity decreased to 92.7±2.6% (25 nL; p<0.05), 91.2±4.2% (50 nL; p<0.05), and 74.8±8.3% (100 nL; p<0.05) of control (n=5). Pressure microinjection of 1.0 mM methysergide (100 nL) into the DRG had no effect on XII neural activity (97.8±4.4% of control; n=5) but did result in a full (95.5±3.9% of control; n=5) or incomplete (decreased to 93.0±28.8% of control; n=2) antagonism of subsequent pressure microinjection of 0.05 mM 5HT (100 nL). We conclude that exogenous 5HT in the DRG decreases XII neural activity. The data further suggest that the DRG may be an important site of 5HT modulation of respiratory drive to the XII motoneurons.


To describe the discharge patterns of phrenic motoneurons (PMNs) statistically, we studied the relation between the interspike interval (ISI) and the order of firing during the inspiratory (ii) phase for discharges of 151 PMNs in 8 unanaesthetized, decerebrate and vagotomized cats. PMNs were classified as early (ii n=25) and late (ii n=59) based on the onset delay (Border line 20 ms). A statistic analysis showed that for all ii the PMNs, the second ISI in the ii phase was significantly shorter than the first. However, for a minority of early ii PMNs (n=18), all having oscillations that were locked to the on-set of the second ii ISI was longer than the first. Thereafter for all the PMNs, the ISIs became continually shorter due to gradually increasing drive. The decrease of ii ISI during the ii phase was analyzed with regression and correlation techniques. With the values of least squares, curve-fitting was done to three types of curves: 1) straight line, 2) exponential curve, and 3) power function. We found that, for a given PMN discharge, the power function was the best fit for the relation between ISI and the order of firing in the ii phase. This power function had the general form

\[ Y = a(t) + b(t)^c \]

where Y is the ISI and X is the order of firing in an ii phase. This relation held for most PMNs, with a correlation coefficient of 0.995 ± 0.007 (mean ± SD, n=105), except for the 18 early ii PMNs whose second ISI was longer than the first. This pattern of time dependent of interval decrease may reflect the properties of the modulatory inputs and the PMN responses. (Supported by N.I.H. Grant HL-27300).

CYCLIC ADENOSINE MONOPHosphATe (CAMP) MEDIATES SHORT-TERM MEMORY WITHIN THE PHRENIC MUSCLE NUCLEUS. Y. Sun, P. G. Wagner and M. S. Dekin*. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, N.J. 08903-0019.

Within the phrenic motor nucleus (PMN), thyrotropin-releasing hormone (TRH) causes presynaptic facilitation via an increase in cAMP while baclofen, a specific GABA_B agonist, has the opposite effect (Sun et al., Neurosci. Abstr., 20:544, 1994). In this study we tested the hypothesis that brief exposure to TRH or baclofen would result in persistent effects on presynaptic cAMP levels resulting in short-term memory within the PMN. Spinal cord slices containing the PMN (C3 to C5) were obtained from 3 to 7 day old rats. Whole cell recordings were made from motoneurons identified by antidromic stimulation of the ventral root. Extracellular postynaptic potentials (EPSPs) were elicited by stimulating the ventral (or lateral) funiculus. A separate pressure ejection electrode was used to apply TRH (10 μM) or baclofen (100 μM). Pressure ejection: A TRH for 30 sec increased the EPSP amplitude while similar application of baclofen reduced the EPSP amplitude. These changes in EPSP amplitude persisted for at least 1 hour. Motoneuron membrane properties, EPSP kinetics, and responsiveness to exogenous L-glutamate were not altered. Bath application of Rp-cAMP, a competitive inhibitor of cAMP at its binding site on protein kinase A, reduced the EPSP amplitude and antagonized the effects of both TRH and baclofen. These data demonstrate that transient exposure to neuromodulators of cAMP have long lasting effects on neurotransmitter release resulting in short-term memory within the PMN. (Supported by the UMDNJ Foundation, Parker B. Francis Foundation, and NIH Grant HL 02314).

PROTEIN KINASES A and C MODULATE THE ACTIVITY OF AN OUTWARD RECTIFYING GABA_B AGONIST ACTIVATED K+ CHANNEL IN CULTURED PREMOTOR RESPIRATORY NEURONS. Y. Sun and M.S. Dekin* Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, N.J. 08903.

Baclofen, a GABA_B agonist, activates a Ba-2+ -excessive outward rectifying K+ channel (Kbca) in premotor respiratory neurons (Wagner and Dekin, J. Neurophysiol. 69:286, 1993). Kbca channels are inhibited by both cAMP and thyrotropin-releasing hormone (TRH). Both 6-bromo-1,3-cAMP and 8-bromo-cAMP cause an increase in the interval between bursts of channel openings resulting in a decrease in nPo. These data suggested that cAMP and TRH shared a common mechanism of action. TRH receptors, however, are usually associated with the activation of PLC (Dekin and Drummond, J. Pharmacol. 96:450, 1989). In this study, therefore, we determined the ability of antagonists of PKA and PKC to protect Kbca channels from TRH mediated inhibition. All recordings were made using the patch clamp technique in the cell-attached configuration. Kbca channels were activated with 100 μM baclofen in the presence of Rp-cAMP (1 μM) or chlorythrine (1 μM). Both Rp-cAMP (not shown) and chlorythrine (not shown) caused a change in the conductance state of the channel from >100 pS (baclofen alone) to <40 pS and an increase in nPo from 0.30 (baclofen alone) to 0.70. The cells were then exposed to TRH (1 μM) in the presence of baclofen and either Rp-cAMP or chlorythrine. Both antagonists prevented the inhibition caused by TRH. These data suggest that not only does TRH produce its effects via PKC, but that the PKA signaling pathway is involved in its effects as well. (Supported by NIH Grant HL02314, the UMDNJ Foundation and Parker B. Francis Foundation).
SYNAPTIC MODULATION OF PHRENIC MOTONEURON EXCITABILITY IN RATS. D.R. McCrimmon*, P. Hayashi & C. Hinrichsen. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611-3008.

Modulation of the excitability of phrenic motoneurons was examined in response to stimulation of their descending inputs. Urethane-anaesthetized Sprague-Dawley rats were paralyzed and their spinal cords transected at C1-2 to remove spontaneous respiratory drive. Descending pathways were activated using a concentric bipolar electrode in the lateral funiculus just caudal to the transection. Single-pulse stimulation of the descending pathway gave rise to a single, short-latency peak of respiratory activity. Double-pulse paired-pulse stimulation intervals (50-200 msec), the second stimulus elicited two peaks. The first peak was identical to that elicited by the first pulse but was followed by the onset of respiratory activity. The amplitude of which was dependent upon the interpulse interval. The shorter the stimulus interval, the greater the amplitude of the second peak. Repetitive stimulation (50-100 Hz, 0.2 ms pulse, 3-120 μA, 10-30 sec trains) of the descending pathway increased the spontaneous activity on the phrenic nerve for about 1 min. Intracellular recording revealed that the increased excitability arose from a depolarization of individual phrenic motoneurons and an increase in synaptic noise. There was also an increase in the amplitude and duration of individual EPSPs in response to single (1 Hz) stimulation of the descending pathway following periods of repetitive stimulation. These data suggest that increases in phrenic motoneuron excitability may contribute to short-term potentiation of breathing. Supported by HL 40336.


These experiments examined the effect of steady state severe hypoxia on the discharge frequency of expiratory motor units in anesthetized cats. Expiratory units were localized from the external oblique musculature while the cats expired against a positive end expiratory pressure (PEEP) of 1.25 cmH2O. Subsequent to unit localization, PEEP was increased and unit activity, inspiratory and expiratory airflow, end-tidal CO2 level and end-tidal CO2 and external oblique electromyographic (EMG) activity were measured for 3-4 minutes during hyperoxia (FeO2 = 1.0) and severe hypoxia (FeO2 = 0.08-0.1). Throughout severe hypoxia end-tidal CO2 was maintained at levels equivalent to that measured during hyperoxia. A total of 15 expiratory motor units were analyzed: 7/15 were active during hyperoxia while 12/15 were active during severe hypoxia. The number of inspirations/expiration (IN/OUT) and discharge frequency (f) of the units increased from a mean (± SD) of 9.5 ± 1.3 and 7.3 ± 3.0 (Hz) during hyperoxia to a mean of 14 ± 2.9 and 10.1 ± 3.1 (Hz) during severe hypoxia, respectively. This increase was paralleled by a significant increase in EMG activity (% of EMGmax) from 19.8 ± 18.0 to 48.1 ± 31.3. Cycle triggered histograms of N/T and f revealed that during both hyperoxia and severe hypoxia the majority of motor units discharged or inhibited during inspiratory and expiratory phases. In addition, the inspiratory impulse rate and discharge frequency for a given interval of the expiratory period was always greater during severe hypoxia compared to hyperoxia. Therefore, activation of the external oblique musculature contributes to lung deflation during the last 70% of the expiratory period during severe hypoxia. In addition, both rate coding and recruitment of motor units contributed to the increase in respiratory motor unit activity.  

ELECTROPHYSIOLOGY OF MEDIATELY RAPID NEURONS IN SLICES AND TISSUE CULTURE. G.B. Robinson* & J.H. Pirsaq. Dept. of Neurology, Yale University, pms 163-166.

Neurons in the medullary raphe project widely to other respiratory nuclei, and contain neurotransmitters which strongly influence breathing. Respiratory activity alters the firing rate of pacemaker neurons in this region and in the ventrolateral medulla (Richerson, 1995; J. Neurophysiol. 73(1):913-44). Neurons in both regions candidates for central respiratory chemoreceptors. Changes in CO2/pH alter the firing rate of these neurons by modulating pacemaker currents, however, the specific currents responsible for the pacemaker potential are unknown. In the present work, patch clamp recordings were made from medullary raphe neurons, and current was used to determine which currents were involved which could potentially be modulated by respiratory acids. Results were compared to data from cultures of microdissected rostral medullary raphe. Neurons firing in response to changes in CO2 were recorded from both preparations. In current clamp, neurons displayed delayed activation, spike frequency adaptation, and maintained hyperpolarization. At high CO2, voltage clamp recordings demonstrated a rapid, transient outward current activated by depolarization from potentials negative to resting potential, as well as inward rectification. These findings suggest that currents in neurons of the medulla include A-current, calcium-activated potassium currents, calcium-activated K' currents, and an inwardly rectifying K' current, each of which can contribute to pacemaker potentials. Any of these currents may play a role in respiratory firing in these neurons. Characterization of the electrophysiological properties of these neurons is essential for determining which ion channels are modulated by respiratory acids, and may help define the cellular basis of respiratory chemoreception. (Supported by the VAMC)

MODELING OF NEURAL MECHANISMS FOR RESPIRATORY PATTERN GENERATION. I.A. Puehle, J. P. F. Patek* P. J. Kroh* & J. S. Schwegler. Neural Computation Group, Boston University, Boston, MA 02215.

The objectives of the research were: (i) to develop computational models of neural mechanisms that provide the respiratory oscillations and specific patterns of respiratory neurons, and (ii) to understand the mechanisms of integration and specific roles of intrinsic properties of respiratory neurons, network properties of their interconnections, and effects of different feedback in the genesis and control of the respiratory pattern. Models of the central respiratory rhythm generator (CRG) were developed in the framework of the network theory of respiratory rhythmo genesis. The models of single respiratory neurons were developed in the Hodgkin-Huxley style and include sodium and a series of potassium and calcium channels. The single neuron models produce the specific firing patterns of respiratory neurons recorded experimentally (i.e. adapting and ramping bursts). Different model versions of the CRG have been considered. They consist of interconnected neurons with different intrinsic properties and peripheral feedback from pulmonary stretch receptors (PSR) and chemoreceptors. The models have the same inspiratory off-switch but different inspiratory-on switch mechanisms. They demonstrate both a stable respiratory rhythm and specific patterns of respiratory neuronal discharges. The models are compared and analyzed on the basis of their performance under normal conditions and under the influence of different perturbations applied to the PSR feedback and to different inputs from different receptors. The simulation results indicate the most plausible network architecture and inspiratory off-switch mechanism. Our models show how the intrinsic biophysical properties of individual respiratory motoneurons of neurons along with the network properties provide the shaping of the specific respiratory patterns.


Transverse medullary slices of mice containing the pre-Bötzinger complex generate spontaneously respiratory rhythmic activity. Respiratory activity can be recorded at all postnatal stages (P0-31) as mass-activity from hypoglossal (XII) roots (Puck et al., 1994; J. Neurophysiol. 72:2338; Ramirez et al. 1995, Eur. J. Neurophysiol. 429: 599). Contains within this slice are the anatomical regions C1 and C2 which are characterized by their alpha-2 adrenergic binding sites (Pflege et al. 1990, J. Comp. Neurol. 297: 253). Using the transverse slice as an in vitro model, we examined the modulatory action of catecholamines on the isolated respiratory network of the developing mouse. At all postnatal stages (P0-18), blockade of alpha-2 adrenergic receptors by the antagonist yohimbine (1-5 μM) led to a decrease in the frequency of rhythmic activity in XII rootlets and in neurons of the pre-Bötzinger complex, an area which is essential for respiratory rhythm generation (Smith et al., 1991, Science 254: 720). Thus, endogenously released adrenaline seems to modulate central respiratory activity. Consistent with an endogenous adrenergic drive was the finding that adrenaline (5 μM) increased the frequency of XII rhythmic activity in neonatal (P0-3) and mature mice (P9-18, n=5) by 114 +/-29% and 83.5+/-15%, respectively. The duration of inspiratory XII bursts was increased in neonates (by 47%) and mature mice (by 61%) which correlated with a reduction in the interburst interval. Modulatory changes in XII rootlet activity were also reflected in neurons recorded intracellularly within the pre-Bötzinger complex. Whole-cell patch recordings from inspiratory neurons indicate that amplitude, slope and duration of depolarizing drive potentials were increased in the presence of 5μM adrenaline. Expiratory neurons were depolarized and rhythmic hyperpolarizations enhanced. The data demonstrate that the adrenergic system plays an important role in central respiratory control.


Raphe neurons influence breathing. Serotonin is known to modulate the cough pattern. This study was motivated by the possibility that raphe neurons may modulate airway sensory information and the respiratory motor pattern during cough. Cough elicited, thoracotomized, paralyzed, phrenic-triggered ventilated cats (8) were used. Extracelular single neuron activity, and phrenic and lumbar neurograms were monitored during fictive cough produced by mechanical stimulation of the intratracheobronchial tree. Up to 6 neurograms were recorded simultaneously with a microdrive unit. Neurons were tested for respiratory modulation of firing rate by cycle-triggered histograms and 2 statistical tests, and for functional linkage to phrenic and lumbar motoneurons by spike-triggered averaging. Of 80 neurons, 10 were respiratory-modulated; 63 had no respiratory modulation. Cells in each category showed either increases, decreases or no change in firing rate during fictive cough. The respiratory modulated cells had only small variations in discharge rate during control respiratory cycles. The results provide evidence for changes in the raphe neuronal network consistent with a modulatory role during coughing. (Supported by NIH HL049813)
373.13   

We have reported favored spots in the spike trains of single neurons that are elements of brain stem cardiorespiratory-related neuronal assemblies identified by their impulse synchrony (FASEB J. 9: A939, 1995). These results motivated a search for multinerve patterns of impuses that repeat more often than expected by chance. Spike trains were recorded in parallel with electrode arrays used for intracellular recording, as well as extracellular recordings of the same neuron in the medulla and the region of the medullar respiratory group of 9 anesthetized (Dial), vagotomized, artificially ventilated cats. Samples of "spontaneous" activity from 4 to 11 simultaneously recorded single neurons were analyzed with the algorithms of Abeles and Gerstein (Neurophysiol. 60:909-918, 1988). The number of different recurring distributed patterns composed of 4 or 5 spikes exceeded (p < 0.01) that expected under the null hypothesis in 12 of 14 data sets. In 6 of 7 data sets, single neurons with previously identified favored patterns were also elements of groups of neurons with "excess" distributed patterns. The results: a) are consistent with the possibility that single neuron patterns may be fragments of distributed impulsive sequences, b) provide another line of evidence for the hypotheses that brain stem neurons operate in coordinated assemblies, and c) encourage the search for coding functions of spike patterns in parallel channels. Supported by NS19184.

373.14   
RESPIRATORY DEPRESSION BY CARBACHOL INJECTION IN THE PONTINE RETICULAR FORMATION IN NEONATAL RATS. M. L. Fund* and W. M. St. John. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

In adult animal, injection of cholinergic agonist, carbachol, in the pontine reticular formation depresses respiratory activity (H. Kusama et al. J. Appl. Physiol. 69: 2280-9). We postulated that the pontine cholinergic mechanism mediated respiratory depression is present in the neonatal animal. Phrenic activity was recorded in decerebrate, paralyzed, ventilated and vagotomized neonatal rats from 4 to 22 days after birth. Small volume (10-60 nl) of carbachol (44-88 mM) was injected in the medial portion of the rostral pontine. The injection of carbachol decreased phasic respiratory activity and respiratory frequency in most animals. The site of injection was confirmed by histology. Result suggests that endogenous cholinergic mechanisms in the medionpons depresses respiratory activity in neonatal animal. This study provide preliminary data for future studies of the nature of the mechanism underlying respiratory depression in developing animal and the possible link of the mechanism to the sudden infant death syndrome.

Integrated Phreonic activity of a 4-day-old rat

Carbachol

1 min

373.15   
AXONAL PROJECTIONS FROM THE PONTINE PNEUMOTAXIC REGION TO THE BÖTZINGER COMPLEX IN CATS. M. Adok*, G. Song, Y. Sato, and I. Kohama. Dept. Physiology, School of Medicine, Sapporo Medical University, Sapporo 900, Japan.

We investigated axonal projections of respiratory and nonrespiratory neurons in the pontine parabrachial - Kölliker-Fuse nuclear complex (NPB-KF, the pneumotaxic region) to a distinct group of respiratory neurons in the brainstem: the respiratory rhythm-generating nuclei, the medulla, in an antidromic mapping technique in chloralose-urethane anesthetized cats. Among 91 respiratory neurons extracellularly recorded in the NPB-KF, 13 neurons (11 inspiratory, 2 phase-symmetric) were found to have axons projecting to the BOTC. Antidromic mapping indicated that those descending axons terminate in the BOTC. Other 55 nonrespiratory neurons were also antidromically activated by electrical stimulation of the BOTC. Recording sites of antidromically activated respiratory and nonrespiratory neurons were distributed in the lateral NPB and KF nuclei. For histological identification of the projections, a small amount of WGA-HRP (30-50 nl, 5%) was injected into the BOTC. A number of retrogradely labeled cells were observed in the NPB-KF and the distribution of labeled cells was similar to that of recording sites. These results suggested that the pathway from the pontine pneumotaxic region to the BOTC is involved in respiratory control.

373.16   

Stimulations of the nasal mucosa strongly influences the cardiovascular and respiratory output. These responses are trigeminally mediated. Recent studies have shown that the parabrachial (PB) and Kölliker-Fuse (KF) nuclei receive prominent somatosensory inputs from the spinal dorsal horn and the spinal trigeminal nucleus. The PB/KF itself has prominent projections to autonomic and respiratory cells groups by which it can strongly modulate their activity. In the present study we investigate whether the PB/KF plays a role in regulating the trigeminally induced cardiovascular and/or respiratory responses. To do so, we stimulated the ethmoidal nerve (EN5) and recorded cardiovascular and respiratory responses before and after injections of the calcium channel blocker CoCl2 into the PB/KF.

Unilateral electrical stimulation of the EN5 resulted in a pronounced depression of respiration (apneusis) and a moderate, but significant bradycardia and pressor response. EN5 stimulations immediately after unilateral injections of CoCl2 into the caudal KF resulted in a significant blockade of the respiratory depression. In contrast, CoCl2 injections into rostrally located parts of the PB/KF showed only weak effects. A recovery of the respiratory response to EN5 stimulation (apneusis) was observed 30 to 60 minutes after the CoCl2 injections. Injections of glutamate into the most effective blocking sites in the KF also caused apneusis, suggesting that the trigeminal input to the KF is mediated by glutamatergic neurotransmission. The cardiovascular responses were also altered after CaCl2 injections. However, whether these effects are statistically significant has to be evaluated by further experiments.

From our data we conclude, that the KF is an obligatory relay site for the trigeminally induced apneusis. Thus, one biological function of somatosensory inputs to the PB/KF might be the mediation of trigeminal nasopharyngeal (protective) reflexes. (Supported by DFG He 1842/3-2)

373.17   
APPLICATION OF IN SITU PCR TECHNIQUE REVEALS THE DISTRIBUTION OF AN INWARD RECTIFYING K+ CHANNEL IN RAT BRAINSTEM. J. W. Roza, K. M. Kofod, and L. Feldman. Systems Neurobiology Laboratory, Department of Neurological Science, UCLA, Los Angeles, CA 90095-1527.

We postulated that an inwardly rectifying K+ channel (IRKc) plays an important role in the modulation of respiratory rhythm in vitro (Johnson et al, Soc. Neurosci Abstr 18: 488, 92). Oligonucleotides identical to the M1 and M2 regions of mouse IRKc1 (Kubo et al, Nature 362: 127, 93) were used as primer and chain reaction (PCR) primers to amplify DNA from neonatal brainstem (Lai et al, Soc Neurosci Abstr 19: 704, 93). A rat IRKc was detected, cloned, and characterized - rat brainstem K+ channel I (RBKSI) (Lai et al, Soc Neurosci Abstr 20: 75, 94). In the present study, our aim is to localize its distribution within the medulla relative to regions averse respiratory control.

Initial tests to study the distribution of mRNA for RBKSI were unsuccessful using conventional in situ hybridization techniques, perhaps due to its low copy gene expression. To increase sensitivity of detection, we employed in situ PCR, using a modified protocol on paraffin embedded sections of adult rat medulla (Nuovo, G. PCR in situ Hybridization: Protocols and Applications. New York: Raven Press, 1992). Supported by NIH Grants HL37941 and NS24742.

373.18   

We examined activity from the ventral medullary surface (VMS) during spontaneous sighs occurring within sleep and waking states in goats, using scattered light imaging procedures. Under sterile surgery, 5 goats were instrumented with electrodes to acquire sleep physiology and diaphragmatic EMG measures, and a miniature video CCD camera coupled to a coherent fiber bundle. The optic probe was placed over a site on the rostral VMS which, when cooled, elicited apnea. Following recovery, all-night sleep recordings were taken, and images were collected at 1/s. Sighs, (sustained inspiratory efforts > 2.5 x interval mean) were preceded by a substantial decline in inspiratory activity for a period of 4-9 sec prior to onset of the sigh. A significant decline in overall activity accompanied the sigh. We conclude that transient respiratory events such as sighs follow alterations in neural activity within the VMS. Supported by HL-22418, NSPS-25719 and NIDR DE 07212. G.P. is supported by a Howard Hughes Medical Institute Predoctoral Fellowship.

We examined activity from the ventral medullary surface (VMS) during spontaneous breathing occurring within sleep and waking states in goats using scattered light imaging procedures. Under sterile surgery, 5 goats were instrumented with electrodes to acquire diaphragmatic EMG and sleep physiology measures, and with a miniaturized video camera and coherent fiber optic probe placed over a site on the cervical VMS which, when cooled, elicited apnea. Following recovery, all-night sleep recordings were taken, images were collected at 1/sec, and diaphragmatic activity was integrated over a long time constant. Slow variation in overall diaphragmatic activity was cross correlated with light reflectance. Relatively synchronous correlations occurred for variance with a period of less than 10 sec. This lag correlation varied considerably across sleep-waking states, and sometimes disappeared entirely during slow-wave sleep. This finding suggests variable links between respiratory effort and the VMS, and underscores the importance of state in the central control of respiration. Supported by HL-22418, USPHS 25739 and NOD DE 07212. G.P. is supported by a Howard Hughes Medical Institute predoctoral fellowship.

Power spectra of gasp-like activity in the phrenic neurogram elicited by chemical activation of neurons in the pre-bötzingker region. N.H. Edelman, and J.A. Neubauer*, Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Progressive brain hypoxia results in a shift from respiratory depression to gasping in peripherally chemoremediated animals. In addition, there is a shift in the autoregressive (AR) spectra of the phrenic neurogram such that peaks appear only in the 30–60 Hz and 60–120 Hz ranges. Recently, we demonstrated that a gasp-like pattern in the phrenic neurogram can be evoked by chemical activation of neurons in the pre-bötzingker (pre-Böt) region. To determine whether the power spectra of such gasp-like activity is similar to that seen during hypoxia-induced gasping, we examined the AR spectra of chemical-induced gasp-like activity. Gasp-like activity was evoked by microinjection of either 0.5-N-monoaminooxyacetic acid (DLH: 10 mM; 21 nl; n = 5) or sodium cyanide (NaCN: 1 mM; 21 nl; n = 3) into the pre-Böt region in chloralose-anesthetized, vagotomized, and paralyzed cats. We also examined the AR spectra of the eupnic pattern immediately prior to microinjection of DLH or NaCN. The AR spectra of the eupnic pattern consisted of a discrete peak in the 30–60 Hz range and another in the 60–120 Hz range. During chemical-induced gasp-like activity there was a downward shift of the 30–60 Hz peak to frequencies < 30 Hz. Prior to cessation of the chemical-induced gasp-like activity, the low frequency power (< 30 Hz) began to shift back towards the eupnic pattern (20–60 Hz range). These findings demonstrate that the changes seen in AR spectra during chemical-induced gasp-like activity in the phrenic neurogram are similar to those seen during hypoxia-induced gasping. Supported by HL16022, HL07467, HL44678, AHA/NF 93-G-37.

In vitro responses of periaqueuctal gray neurons to hypoxia and hypercapnia. J.M. Kramer*, P.C. Nolan and T.G. Waldrop*, Dept of Physiology & Biophysics, Kinesiology, Neuroscience Program and College of Medicine, Univ of Illinois, Urbana, IL 61801.

Previous studies from this laboratory have demonstrated that hypoxia and hypercapnia stimulate neurons in the ventrolateral medulla and in the caudal hypothalamus both in vivo and in vitro. In addition, we have recently shown that central hypobalamic neurons which respond to hypoxia and possess a cardiorespiratory related discharge project to the periaqueuctal gray (PAG). The purpose of the present study was to determine if neurons in the PAG are responsive to hypoxia and hypercapnic stimuli in the absence of input from peripheral chemoreceptors and other brain regions. Brain slices (400–500 μm) containing the PAG were obtained from Sprague-Dawley rats and placed in an interface chamber perfused with nutrient medium bubbled with 95% O2/5% CO2. Single unit, extracellular responses of PAG neurons to hypoxia (10%/5% O2/5% N2) and to hypercapnia (7% CO2/93% O2) delivered for 60–120 sec and 3 min., respectively, were recorded. Hypoxia altered the discharge rate in 73% of the neurons tested, only 9% of the neurons responded to hypercapnia. Most (85%) of the hypoxia sensitive neurons were stimulated by hypoxia. Perfusion of the slices with a synaptic blockade medium (low Ca2+/high Mg2+) did not block the excitatory responses to hypoxia. These findings demonstrate that PAG neurons have an inherent response to hypoxia. These results and our prior findings are consistent with a hypothalamic-PAG-modulatory pathway involved in the mediation of cardiorespiratory responses to hypoxia. (Supported by NIH grants HL38720 and HL06296).

Intrinsinc effects on membrane potential and input resistance of chemical hypoxia on cultured neurons (Supported by M. Akyuz, D.C. Solomon, J.M. Kramer*). Department of Physiology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

In the face of synaptic insult, central neurons will decrease activity as a means of conserving energy and avoiding cell death. Recent work in vivo has also demonstrated substantial population of neurons that are excited by hypoxia. The goal of this study was to determine the intrinsic effects of chemical hypoxia on membrane potential (Vm) and input resistance (Rin) on cultured neurons from the RVLM, a brainstem area containing neurons important to central cardiovascular and respiratory control. The studies were performed on neonatal rat neurons (2-3 days old) plated on astrocyte monolayers using the whole cell perforated patch clamp technique. The cells were perfused in either mock-CSF or mock-CSF containing low Ca2+ (0.5 mM) and high Mg2+ (4.0 mM) to block synaptic transmission and were equilibrated with 95% O2/5% CO2. Sodium cyanide (NaCN, 1.10 mM) was given as a bolus into the perfusion line and the Vm, Rin, and firing frequency (FF) were analyzed in 18 neurons. Of these 18 neurons, 7/18 (39%) were hyperpolarized and decreased FF, while 11/18 (61%) were depolarized and increased FF with NaCN. These responses were unaffected by synaptic blockade. Excitation was associated with a decrease in Rin while depression was associated with an increase in Rin. In all cases responses were repeatable and reversible. Thus, cells cultured from the RVLM show responses that are intrinsic and not sympathetically mediated, similar to those demonstrated in vivo. These results further suggest the existence of a population of neurons that are excited by hypoxia and that may act as central O2 sensors. (Supported by HL16022 and HL07467).

In vitro responses of periaqueuctal gray neurons to hypoxia and hypercapnia. J.M. Kramer*, P.C. Nolan and T.G. Waldrop*, Dept of Physiology & Biophysics, Kinesiology, Neuroscience Program and College of Medicine, Univ of Illinois, Urbana, IL 61801.

In vitro responses of periaqueuctal gray neurons to hypoxia and hypercapnia. J.M. Kramer*, P.C. Nolan and T.G. Waldrop*, Dept of Physiology & Biophysics, Kinesiology, Neuroscience Program and College of Medicine, Univ of Illinois, Urbana, IL 61801.

In vitro responses of periaqueuctal gray neurons to hypoxia and hypercapnia. J.M. Kramer*, P.C. Nolan and T.G. Waldrop*, Dept of Physiology & Biophysics, Kinesiology, Neuroscience Program and College of Medicine, Univ of Illinois, Urbana, IL 61801.

The response of chemoreceptor neurons to hypercapnic stress was examined using exposure of anesthetized rats (n=8) to 5% CO₂ for 60 min induced activation of the c-fos gene, expressed as fos like immunoreactive protein (flios), within brainstem, mesencephalic dopamine containing regions. Co-localization studies of tyrosine hydroxylase (TH) and fos protein revealed that the majority of the brainstem TC-containing neurons expressed fos immunoreactivity. Numerous double labeled neurons were found in the ventrolateral medullary reticular formation (ATC1 cell group), in the ventrolateral subnuclei of nucleus tractus solitarius (A2/C2), TH-containing neurons in the ventral lateral pontine nuclei (A5 cell group), and also showed flios immunoreactivity in response to hypercapnic loading. These results suggest that chemoreceptor-containing neurons are part of the neuronal network involved in the response to CO₂. Supported by HL-25830 and HL-50627.


The rostral ventrolateral medulla (RVLM) has been implicated in the central CO₂ chemosensory regulation of cardiovascular and respiratory function. In this investigation, the effect of acidic mock cerebrospinal fluid at the RVLM was evaluated for c-fos immunoreactivity. c-fos was found in small interneurons embedded in the marginal glia(MG) of the caudal VLM and RVLM. c-fos positive cells were also detected in nucleus retroambiguus, lateral reticular nucleus, the area postrema, cranial nerve nuclei, and the n. tractus solitarius of the caudal medulla. Rosally, positively stained cells were located in the nucleus of the tractus solitarius, medullary parabrachial nucleus, olivary nucleus, cerebellar nuclei, pontine nucleus, the reticulotegmental nucleus of the pons, and the interpeduncular, red and oculumotor nuclei of the midbrain. These data demonstrate that acidic mock CSF applied to the RVLM induces c-fos in several respiratory related nuclei and indicates that multiple sites of respiratory chemosensitivity may exist within the brainstem. Supported by ONR Grant No. N00014-94-1-0523.
739.1
ACETHOLAMINE INCREASES INTRACELLULAR CALCIUM OF CULTURED CAT CAROTID BODY CELLS. M. Shirahata*, J.S.K. Sham, and R.S. Findlay. Departments of Environmental Health Sciences & Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Acetholamine (AcH) is present in glomus cells of the carotid body (CB). AcH receptors are located on glomus cells as well as carotid sinus nerve (CSN). Exogenous AcH from the CSN increased CSN neuronal activity and dopamine release. Perfusion of the CB with blockers of AcH receptors inhibits neural response of the CB to hypoxia. These data suggest AcH may have multiple actions in the CB. As one of the steps to elucidate possible roles of AcH on the CB in chemoreception, we examined the effect of AcH on intracellular calcium concentration ([Ca++]i) of cultured CB cells with microfluorometric technique using indo-1. CB cells were loaded with indo-1 for 1 hour, and extracellular solutions were removed by superfusion of Krebs solutions for 45 min. Experiments were performed at 37°C, and cells were continuously superfused with Krebs solution or Krebs solution containing pharmacological agents. All solutions were equilibrated with 5% CO2/16% O2. Ca++, was measured from clusters. AcH (1-100 μM) increased [Ca++]i and appreciably increased 80% of clusters tested responded to AcH. Nicotine mimicked AcH effect, and pilocarpine occasionally caused very small increase in Ca++. Increase in Ca++ by AcH was not blocked by caffeine which depletes Ca++ from intracellular storage sites. A type-calcium channel blocker, nifedipine, did not block the effect of AcH. The results suggest that AcH increases Ca++ of cultured CB cells through activation of nicotinic channels.

Supported by HL 47044, HL 50712, and HL 52652.

739.2

Our previous data using in vitro culturing states that involvement of voltage sensitive calcium channels (VSCC) for the CB response to hypoxia. We have been culturing CB cells from adult cats, and the cultured glomus cells released dopamine in response to hypoxia. These data are well fit a current hypothesis of carotid body (CB) chemoreception. That is, hypoxia depolarizes glomus cells followed by an activation of VSCC. We analyzed this hypothesis using microfluorometric technique to monitor intracellular calcium ([Ca++]i) of glomus cells and triggers neurotransmitter release. We tested the following possibilities if the cat glomus cells are depolarized during hypoxia, and if Ca++ increases during hypoxia. The CB cells cultured on glass coverslips were superfused with Krebs solution equilibrated with 5% CO2/16% O2, 5% CO2/16% O2, or 5% CO2/16% O2, in the recording chamber was monitored continuously. Membrane potentials (Eh) of glomus cells were measured with patch clamp techniques. Eh depolarized from -54±3 mV during hypoxia to -26±5 mV during hypoxia (20-30 mm, 4°C). Sheath cells and other non-glomus cells in the cluster were not significantly depolarized during hypoxia. Ca++ of clusters was measured with microfluorometric technique using indo-1, and it did not show significant changes during hypoxia. On the other hand, application of 100 mM K+ clearly increased Eh, suggesting the presence of functioning VSCC. Data suggest that under these experimental conditions depolarization of glomus cells during hypoxia may not be enough to fully activate VSCC. Supported by HL 47044, HL 50712, and HL 52652.

739.3
CATECHOLAMINE RELEASE FROM RAT CAROTID BODY ENDOPLASMIC RETICULUM IN NONOXIC AND HYPOXIC ENVIROMENTS. A. Janczak and G. Bistro* Dept. of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

63 Chronic hypoxia in vivo produces adaptive changes in the chemoreceptive carotid body (CB) resulting in time-dependent modifications of transmitter function. Several adaptations to hypoxia have been identified in CB Ch-ergic receptors (glomerular cells) including cell hypertrophy, changes in ion channel expression and neurotransmitter function. By exposing cultures of dissociated Wistar rat CB to different O2 tensions, we have uncovered some plastic properties of glomus cells that are likely due to direct effects of hypoxia (P.N.A.S. 89: 9469, J. Neurosci. 26: 485, J. Neurosci. 15: 3192). In this study we begin an investigation of the neurotransmitter status of these cultures by measuring dopamine (DA) release using HPLC (with electrochemical detection). Both acute hypoxia (6 or 10% O2) and high K+ medium stimulated DA release from control cultures grown in normoxia for 1-2 weeks. Compared to controls, basal DA release was consistently higher in chronically hypoxic cultures. Further, K+-evoked DA release (pmsl/1000 glomus cells) was significantly higher in chronically hypoxic compared to control cultures after 2 weeks in vitro. We are investigating whether these modifications in neurotransmitter function can be correlated with other known physiological and biochemical changes that have been identified in these chemoreceptors after chronic hypoxia. Supported by MRC Canada and the Heart and Stroke Foundation of Ontario.

739.4
CARBONIC ANHYDRASE AND CO2 CHEMORECEPTION IN FROG OLFACTORY RECEPTOR NEURONS. E.L. Coe* and R.P. Smith. Department of Biology, Allegheny College, Meadville, PA 16335

There is substantial evidence that the enzyme Carbonic Anhydrase (CA) plays an important role in the detection of CO2 by central (Coates, U. and Nattie, 1991) and peripheral (Hanson, N., and Tomaras, P., 1981; Erich et al., 1990; and Leiter, 1994) carotid body chemoreceptors (Lahir et al., 1993). The objective of this study was to present preliminary data which suggest the olfactory CA neurons in the frog olfactory bulbs also use CA to detect changes in CO2.

To test this hypothesis, olfactory generator potentials were recorded from the ventral epithelium of bullfrogs using glass electrodes (tip diameter = 15-25 μm). Olfactory receptor neurons were tested to obtain their responses to second and fourth power of 5% CO2 and amyl acetate before and after (up to 90 min) topical CA inhibition with acetazolamide (10M) and before and after application of an inactive acetazolamide analog (30 μM).

In 52 bullfrogs, 1222 sites on the ventral epithelium were checked resulting in 23 locations that exhibited a response to CO2. It was found that CA inhibition caused a 50% reduction in sensitivity to CO2, and that sensitivity recovered when CO2 was available in the absence of CO2. The olfactory CA neurons responded to either CO2 or amyl acetate. These results indicate that the olfactory CA neurons respond to CO2 by releasing CO2-sensitive neurotransmitter in ventral epithelium.

Supported by NSF grant # IBN 94-09269

Although previous studies have demonstrated that CA cells rapidly degenerate in response to hypoxia in type I cells during patch-clamp experiments, it has nonetheless been suggested that CA-cell function in these cells is not regulated by cyclic AMP-initiated protein phosphorylation. [J. Neurochem. 57: 1992-2000, 1991]. In the present study, the patch-clamp and artificial ATP treatments were applied to isolated rabbit carotid body A (PKa) cells in patch pipettes markedley slowed the rundown and significantly enhanced the peak values of sustained inward (L-type) CA currents recorded in freshly dissociated chemosensory cells, an effect which was mimicked by o-methyl acid, a protein phosphatase inhibitor. Rat-ionic imaging revealed that agents which increase cAMP in carotid body type I cells (forskolin, 10 μM; adenosine, 100 μM) also potentiate hypoxia-evoked intracellular CA responses. Finally, a transfected cell-permeant PKA inhibitor, Rp-cAMP, dephosphorylates CA-cholinomimetic (synthesized from H-tyrosine) release evoked from superfused carotid body cells. The current results are consistent with the hypothesis that hypoxia, which elevates cAMP in type I cells, initiates a cascade of events resulting in elevated intracellular Ca²⁺ levels and enhanced CA release. Supported by USPHS grants NS12636 and NS07938.

RESPIRATORY REGULATION: DEVELOPMENTAL MECHANISMS

740.1


The transverse pre-Bötzinger complex (pBC) of mice has recently been introduced as an in vitro system to study the postnatal development of the respiratory system in mice (Funk et al. 1994, J. Neurophysiol. 72: 2538; Ramirez et al. 1995, Eur. J. Physiol. 426: 599). This technique allows the study of isolated postnatal stages (P0-31) spontaneous respiratory activity that can be recorded as mass activity from hypoglossal (XII) roosters. XII roosters in mice are in phase with inspiration and with generation of phase-relationships of simultaneously recorded rhythmic neurons in the pBC, an area critical for respiratory rhythm generation. During the first 3 postnatal weeks, the duration of XII bursts remained unaltered. However, the phases of XII bursts changed from desynchronized (P1-7) to well-synchronized (P8-22), correlated with a significant decrease (p<0.008) in the rise of integrated bursts (-43.5±11.8) during the coupling between burst and XII bursts and those to XII roosters changed during postnatal development, being 1:1 in a neonate and on average 3:1 in a mature mouse. No obvious developmental change occurred in generation mechanisms at all examined postnatal stages (P0-31) rhythmic activity was abolished by 10μM of the non-NMDA antagonist CNQX. Rhythmicity was maintained in the presence of glycine (sturanine, 0.2-20μM) and GABAergic (bicuculline, 50μM). Although inhibitory synaptic mechanisms seem not essential for rhythm generation, they are important for pattern generation. In XII roosters blockade of inhibitory mechanisms caused an increase in frequency and burst amplitude and a decrease in burst duration. In the pBC blockade of inhibitory synapses increased rhythmic drive potentials and intraburst spike frequency of inspiratory neurons. The phasic hyperpolarization generated in expiratory neurons was decreased in the presence of strychnine. Following blockade of both glycineic and GABAergic inhibition expiratory neurons were found to discharge in phase with inspiration.

740.3


The response of the mammalian respiratory system to hypoxia changes dramatically during postnatal development and several different sites within the respiratory system have been proposed to be responsible for this phenomenon. We examined developmental changes in the hypoxic response of the isolated respiratory network using a rhythmically active mediolateral slice preparation of mice (Funk et al. 1994, J. Neurophysiol. 72: 2538; Ramirez et al. 1995, Eur. J. Physiol. 426: 599). Respiratory rhythmic activity at all postnatal stages (P0-31) studied was decreased extracellularly and intracellularly from pre-Bötzinger complex neurons. Hypoxia, maintained for 20 - 30 minutes, was induced by replacing carbogenated artificial CSF with one containing a mixture of 5% N₂ and 5% CO₂ in the presence of normoxic mice (P0-15) hypoxia induced a significant decrease in frequency (34.7% ± 5.6) which never led to a cessation of rhythmic activity. Decrease in amplitude (-5.4% ± 2.8) and rise time (+58.3% ± 31.0) of XII bursts were not significantly affected by hypoxia. In contrast, in mature animals (P7-14, n=9) hypoxia caused an initial increase in the frequency of XII activity which was accompanied by a burst duration decrease (45.5% ± 12.2), amplitude decrease (85.8% ± 26.9) and rise time (298% ± 137) of XII bursts. We refer to this initial phase as augmentation. Augmentation was followed by depression of rhythmic activity and XII burst amplitude and frequency (20.2% ± 9.4), but also in burst duration (-9.1% ± 8.2), amplitude (-21.2% ± 8.5) and rise (5.5% ± 18). Depression led to abolition of rhythmic XII activity (central apnea). Inspiratory neurons to XII were initially depolarized and subsequently hyperpolarized. Expiratory neurons were only hyperpolarized. Under normoxic conditions both groups showed complete recovery. Our results indicate that the isolated central network for respiration undergoes dramatic maturational changes in its hypoxic response which can now be analyzed under in vitro conditions.

740.4

GLOMUS CELL EXCITABILITY AND CAROTID BODY REFLEXES IN NEONATAL AND ADULT RATS ACCLIMATIZED TO CHRONIC HYPOXIA. S.C. Hempelmann, F.L. Powell, and S.M. Algesheimer. Dep. of Medicine, University of California, San Diego, La Jolla, CA, 92093.

Chronic hypoxia (CHX) for several days to weeks increases the acute hypoxic ventilatory response (HVR) in adult rats, but has variable effects in neonatal rats. To study the effect of CHX and carotid body glomus cell Na⁺ and K⁺ currents in chronically normoxic (CON, PO₂=140 mmHg) and chronically hypoxic (CHX, PO₂=80 mmHg) adult (24-30 wks old) and neonatal rats (1 week old). Rats were acclimatized to CON or CHX for 3-4 weeks. The effect of CHX on reflexes for inspiratory and expiratory glomus cell Na⁺ and K⁺ currents were measured in isolated carotid body glomus cells using whole cell patch clamp techniques. In neonates, CHX decreased Na⁺ current (28% ± 7.4, n=9) and increased K⁺ current (19% ± 4.3, n=9). The acute adult rat glomus cell Na⁺ current decreased significantly (42% ± 7.1, n=7). These results are consistent with increased glomus cell excitability in both adults and neonates after CHX, and suggest that blunting of the HVR in neonates is a CNS effect of CHX. Supported by NIH HL-17731.
740.5

RESPIRATORY SUGGESTIONS AFFECTS THE TIMING AND PATTERN OF RESPIRATORY BURST IN RESPIRATORY BURST IN THE NEONATAL RAT IN VITRO: BRAINSTEM SPARE, CORD PREPARATION. B L. ELLERENBERG* and E. F. SMITH. Department of Anatomy and Neurobiology, Dalhousie University, Halifax N.S., Canada, B3H 4H7.

Anatomical evidence suggests a role for cholecystokinin (CCK) in respiratory control (Ellerenberg et al., J. Chem. Neuro., 3:375, 1952). CCK receptors can directly affect neuronal membrane conductances or act through a variety of second messenger systems. We examined the role of CCK-A and -B receptors on respiratory pattern in the in vitro neonatal rat brainstem-spinal cord. Long Evans rats (0-3 days old) were anesthetized with ether and the caudal thoracic spinal cord was removed, divided and placed in flowing artificial CSF solution (pH 7.4, 27°C). Respiratory burst activity was recorded extracellularly from C5 ventral roots. Activation of CCK-A receptors by bath application of CCK8-Sulfated (CCK8-S) doubled respiratory burst frequency at a concentration of 30 and 60 nM CCK-S, and 120 nM CCK8-S burst duration became prolonged with long augmenting and decrementing phases. There was little or no change in burst amplitude or tonic activity. Bath application of similar volumes and concentrations of the CCK-B agonist, CCK8-Unsulfated, did not produce these effects. The CCK-A specific agonist, lorglumide (600 nM) blunted or abolished responses to all concentrations of CCK-S. These results suggest that CCK-A receptors can alter rate and pattern of respiratory burst discharge by acting primarily within the medullary respiratory network and not directly on respiratory motoneurons.

Supported by MRC grants MT12212 (HHE) and MT16162 (FMS). FMS is a Scholar of the Heart and Stroke Foundation of Canada.

740.7


Serotonin (5-HT) exhibits powerful modulatory central control during ontogenesis of the mammalian brainstem respiratory network. In addition, it has potential trophic effects on development of synaptic interaction. We mapped for 5-HT-Immunoreactivity (5-HT-IR) in the ventral respiratory group (VRG) including the pre-Bötzinger-Complex in pre- and postnatal rat brainstems. 5-HT-IR cell bodies of the local caudal raphe nuclei appeared at embryonal day 14 (E 14), terminals with varicosities were detected in the VRG at E 17, the number and density of 5-HT-IR fibres as well as fibre branchings within the VRG increased rapidly during the following three weeks reaching adult levels at postnatal day 14 (P 14). In contrast, 5-HT-IR terminal varicosities were densely distributed already at E16-17 and remained at a constant level of density during maturation. Therefore, local serotonergic innervation appears to be present during the entire period of pre- and postnatal development of medullary respiratory centers. The increase in 5-HT-IR fibre density may reflect the development of synaptic interaction within the respiratory network.

Supported by DFG.

740.9


In adult animals, the discharge of the phrenic nerve, representing central inspiratory activity contains two components termed medium frequency oscillations (MFO), in the range of 20-30 Hz. and high frequency oscillations (HFO), in the range of 50-100 Hz. MFOs are concentrated in the cat and piglets, while MFOs were present in newborn animals, HFOs appeared later in the development and were therefore considered an index of maturation of the respiratory system. It has also been found in kittens that the number and density of 5-HT-IR fibres as well as fibre branchings within the VRG increased rapidly during the following three weeks reaching adult levels at postnatal day 14 (P 14). In contrast, 5-HT-IR terminal varicosities were densely distributed already at E16-17 and remained at a constant level of density during maturation. Therefore, local serotonergic innervation appears to be present during the entire period of pre- and postnatal development of medullary respiratory centers. The increase in 5-HT-IR fibre density may reflect the development of synaptic interaction within the respiratory network.

Supported by DFG.

740.6


To investigate the neuronal mechanisms of spontaneous respiratory rhythm generation in the brainstem, we analyzed the periodicity of burst intervals in brainstem slice preparations of neonatal rats (1 - 7 days). Transverse slices (750 µm thick rostral to the obex, pre-obs region) were prepared. Spontaneous burst discharges were extracellularly recorded from the hypoglossal nucleus (XII n.) and/or the ventrolateral region including the pre-Bötzinger complex. The onset time of each burst was detected with a Schmitt trigger circuit. During the recording (20 - 60 min), burst to burst intervals (T) were automatically determined by means of a digital clock counter. The bursting rhythm of XII n. steadily continued for a few hours in the seven days after birth preparation, with the mean interval of 7.5 ± 0.2 and coefficient of variance of 0.16. We compared these statistical rhythm properties between of medullary neuronal discharges and of CS rosette nerve discharges obtained from the brainstem and spinal cord preparation (previously reported). The results showed that the rhythm from a single medullary slice had similar statistical properties to that from the brainstem and spinal cord preparation. It is suggested that the pre-Bötzinger region is a sufficient subsystem for generating the respiratory rhythmic burst in the brainstem and spinal cord in neonatal rats.

Supported by MRC grants MT12212 (HHE) and MT16162 (FMS). FMS is a Scholar of the Heart and Stroke Foundation of Canada.

740.8


During the second and third trimesters of rat gestation, phrenic motoneurons grow out from the cervical spinal cord (C5-C5) through the developing thoracic cavity to innervate the diaphragm in a highly stereotypic manner. Toward understanding some of the molecular events associated with these processes, we examined the pattern of expression of growth associated protein (GAP-43) and polysynaptized neural cell adhesion molecule (PSA-NCAM) in the phrenic nerve and diaphragm (E11-P0). Immunohistochemical detection of GAP-43, PSA-NCAM and NCAM was performed using anti-GAP-43 MAB (Suga, 12E3 MAB (T. Saka) and polysynaptized NCAM (E. Bock), respectively.

PSA-NCAM expression was restricted localized along growing phrenic axons at three major directional decision areas: i) at the pleural plexus, where phrenic and brachial axons diverge; ii) at the primary branching site within the diaphragm, where three distinct branches arise; iii) along intermuscular branches, where axons separate from the nerve trunk to innervate developing myotubes. The expression of PSA-NCAM in the phrenic nerve thus correlates spatiotemporal with spinales-innervation and motoneuron pool-specific guidance. There was also a clear spatiotemporal distribution of PSA-NCAM in developing diaphragmatic myotubes which was correlated with the regional innervation and intermuscular branching of the phrenic nerve and possibly secondary myogenesis. Functional studies of the role of PSA-NCAM were performed by looking at perturbations induced by cleavage of the PSA moiety from the NCAM molecule via in-situ recombinant production of the enzyme endo-neuraminidase (U. Rutschhauser). Funded by MRC and AHPMR.

740.10


To determine whether the severity or the pattern of hypoxia is important to the induction of seizure electrocorticography (ECOg) with hypoxia and gasping respiration, cortical electrodes and an arterial cannula were implanted chronically in 16 piglets aged 10-22 d. In 48 studies, piglets were exposed to rapid-onset hypoxia of 5%, 8% or 10% for 30 minutes. Animals were awake, moving freely and hypoxia was either continuous (CH) or repetitive (RH), lasting 21 min in total. RH comprised 7 x 3-min intervals alternating with 3-min recovery epochs in air. ECOg & respiratory responses were filtered and digitally acquired, to permit spectral analysis of the ECOg signals. The ECOg frequency spectrum shifted towards the delta band during hypoxia. Seizures and ECOg depression occurred repeatedly in 6 of 12 piglets exposed to 6% RH, whereas a single event occurred in 2 of 8 piglets during 6% CH. Cortical depression was more marked when hypoxia occurred in brief, repeated cycles as compared to CH of equivalent severity and duration, suggesting particular sensitivity of the cortex to rapid changes in oxygen tension at the onset of hypoxia. Respiratory and metabolic adjustments in CH may aid in the recovery from ECOg events.

Supported by NHLBI HL-35939 (USA, IRM), the Montreal Children's Hospital Resa. Inst. (KAW); the Foundation for the Study of Infant Deaths (UK) & The Wellcome Trust (UK, CSB).

In goats, T3 to T6 increases spinal serotonin (5-HT) immunoreactivity in rostral thoracic segments (FAESB J 6: 1507,1992). To determine if changes in other descending modulatory systems occur following TDR, dopamine (DA), noradrenalin (NE) and 5-HT concentrations were assessed via HPLC in the cervical and thoracic spinal cord dorsal horns, following sham surgery (n=4), and 3-12 months following TDR (n=7). Following barbiturate overdose, spinal cords were harvested and frozen for HPLC analysis of homogenized samples. Compared to sham and control animals, TDR increased DA in the cervical (C3-Cc, 155%), rostral thoracic (Tc-T5, 190%) and caudal thoracic (T5-T7, 220%) spinal cord (all p < 0.0001). Smaller NE elevations were observed in these regions (28-50%), p = 0.04% between T3-T4, but only 16% between T3-T5; 5-HT concentrations were elevated 68% in the cervical region (p < 0.002). The results indicate that TDR increases spinal concentrations of neurotransmitters associated with descending modulation of spinal sensory-motor integration. Since effects were observed in both the denervated thoracic region, and in cervical regions associated with the phrenic motor nucleus, it is possible that changes in monoaminergic brainstem-spinal cord pathways play a role in the functional deficits and recovery of ventilatory control that are observed in goats following TDR (NIH HL 36780).


In goats, TDR from T3 to T5 increases spinal serotonin (5-HT) immunoreactivity in rostral thoracic segments (FAESB J 6: 1507,1992). To determine if changes in other descending modulatory systems occur following TDR, dopamine (DA), noradrenalin (NE) and 5-HT concentrations were assessed via HPLC in the cervical and thoracic spinal cord dorsal horns, following sham surgery (n=4), and 3-12 months following TDR (n=7). Following barbiturate overdose, spinal cords were harvested and frozen for HPLC analysis of homogenized samples. Compared to sham and control animals, TDR increased DA in the cervical (C3-Cc, 155%), rostral thoracic (Tc-T5, 190%) and caudal thoracic (T5-T7, 220%) spinal cord (all p < 0.0001). Smaller NE elevations were observed in these regions (28-50%), p = 0.04% between T3-T4, but only 16% between T3-T5; 5-HT concentrations were elevated 68% in the cervical region (p < 0.002). The results indicate that TDR increases spinal concentrations of neurotransmitters associated with descending modulation of spinal sensory-motor integration. Since effects were observed in both the denervated thoracic region, and in cervical regions associated with the phrenic motor nucleus, it is possible that changes in monoaminergic brainstem-spinal cord pathways play a role in the functional deficits and recovery of ventilatory control that are observed in goats following TDR (NIH HL 36780).

741.4 HYDRAULIC DECREASES APNEA-INDEX IN SPONTANEOUSLY HYPERTENSIVE RATS. J.A. Fagouli, R.C. Lefkopoulos, and J.B. Robinson, Dept. of Medicine and Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Spontaneous apnea events have been described in normotensive Sprague-Dawley and Wistar rats, and compared to central apneas in man. In view of the epidemiological association between apnea and hypertension in man, we hypothesized that spontaneously hypertensive (SHR) Wistar-derived rats would exhibit sleep related apnea which could be suppressed acutely by administration of various agonists and antagonists. We found that closure of 2 mg/kg of hydralazine to 9 adult male SHR rats implanted with EEG and EMG electrodes for sleep/wake scoring and placed in unrestrained body plethysmographs for respiratory monitoring. For each rat, sleep and respiratory activity were polygraphically recorded for 6 hours on 2 different days. Immediately prior to each recording, saline or drug was given by intraperitoneal injection. We defined apnea as cessation of respiratory effort for at least 2.5 seconds and expressed apnea indexes (AIs) as apneas per hour. The effect of hydralazine, documented on a different day by tail-cuff plethysmography, was significant (p<0.001) and consistent for 6 hours after injection: baseline = 219±4.2, 2Hrs = 157±6.0, 4Hrs = 167±8.4, 6Hrs = 169±6.73 mmHg. During NE sleep, Al decreased from 12.9±1.4 for saline injection to 5.1±1.3 following hydralazine (p < 0.05). In contrast, during REM sleep, Al did not decrease (p > 0.05); 14.9±3.0 after saline injection, 14.0±4.1 after hydralazine. The response to hydralazine was not affected by the presence or absence of an augmented breathing pattern preceding the apnea (p > 2). We conclude that SHR rats exhibit frequent apneas during most of stages of sleep. Further, we conclude that acute lowering of mean blood pressure significantly reduces apnea expression in NE sleep, not in REM sleep. We speculate that baroreflexes may play a state-dependent role in apnea genesis in the rat.

741.5 ROLE OF THE FASTIGIAL NUCLEUS (FN) ON VAGALLY MEDIATED RESPIRATORY RESPONSES IN CATS. F. Xu and D.L. Frazier*. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

The FN within the cerebellum has been shown to modify the respiratory responses to hypoxia and hypercapnia (Xu et al., 1992). The primary goal of this study was to determine the involvement of the FN in the respiratory responses evoked by activation of vagal afferents. Experiments were performed on chronically vagotomized, chronically paralyzed, and artificially ventilated cats with an occipital exposure of the cerebellum. The phrenic neurogram was used as an index of respiratory neuronal activity. Inspiratory output (partial pressures of CO2 and O2) were compared before and after bilateral lesions (thermal) of the FN. We observed that both thermal lesions resulted in the abolition of vagal afferent activation of respiratory neuronal responses elicited by activation of non-nociceptive vagal afferents. (NIH PO1 40369)

741.6 CENTRAL COMMAND INCREASES TOTAL LUNG RESISTANCE IN DECREBRATE CATS. A.M. Atoek, A.C. Borghem*, and M.P. Kaufman, Division of Cardiovascular Medicine, University of California, Davis, CA 95616.

The neural mechanisms mediating the airway dilatation evoked during exercise are not completely understood. Two mechanisms believed to play important roles in the cardiovascular and respiratory adjustments are the muscle reflex and central command. For example, activation of group III and IV endings in the skeletal muscles reflexly dilates the airways and increases ventilation, heart rate and arterial pressure. Similarly, inactivation of central locomotor regions of the spinal cord increases ventilation, heart rate and arterial pressure. Whether central command plays a role in the control of airway caliber during exercise is not known. Therefore, we tested the hypothesis that stimulation of the mesencephalic locomotor region (MLR) in paralysed, de cerebrate cats increased total lung resistance (TLR). Electrical stimulation of the MLR increased TLR (97±7.2 to 3.7±3.1 cm H2O/l/s, p<0.05; n=7). Similarly, microiontophoretic application of a GABA antagonist (100-800 nl of 5 mg/ml picrotoxin) to the MLR also increased TLR (24±1.4 to 30±8.6 cm H2O/l/s, p<0.05; n=7). In one site, picrotoxin decreased TLR. All of these effects were evoked by electrical stimulation of the MLR were accompanied by an increase in ventral root discharge and arterial pressure. In 10 cats, the laryngeal nerve was electrically stimulated with current of 90 to 110 µA. Changes in airway resistance evoked by electrical stimulation of the MLR were not accompanied by changes in laryngeal nerve discharge and arterial pressure. This study suggests that a central command is involved in mediating the airway dilatation during exercise. Instead, this dilatation may be mediated by peripheral mechanisms, one of which is the muscle reflex. Supported by NIH HL40910.
741.7

Inhibitory effects upon breathing follow SLN stimulation are widely known but similar effects upon swallowing, or any other structures, have not been described. In postmortem anesthetized cats and rabbits observations were made by recording pressure changes in the separated lung. The mechanical changes obtained from a separated vocal cord, tying a thread at a piece cut from the anterior medial part of the thyroid cartilage and connecting it to a piezoelectric (Nodd 1979) Single shocks to the SLN 0.1 max (threshold about 4 maxal 4-5V) elicited phasic laryngeal pressure reductions, 70 maxal latencies, or tension increases of the Vocal cord and similar characteristics. Sometimes the responses were followed by changes in the opposite direction that disappeared or gradually appeared turning a monophasic into a biphasic response. 2-4 trains (10-20 Hz) appeared for 5-30 sec, produced potentiation of the response to single shocks lasting for minutes depending on the duration of the trains. Similar long lasting potentiation occurred after a long duration pressure pulse to the larynx. It is concluded that affects in the SLN have excitatory effects upon the pregestructures of the vocal cords apart from its inhibitory action upon breathing.

741.9
VESTIBULAR EFFECTS ON THE UPPER AIRWAY. Alan D. Miller* and Martina S. Sinaia. Lab. Neurophysiology. The Rockefeller University, New York, NY 10021.

Activation of the vestibular nerve produces reflex responses recorded from respiratory muscle nerves of the thorax and abdomen (Yates, Jakus, Miller, Brain Res. 629: 209-217, 1993). In order to better understand the functional significance of vestibulo-respiratory reflexes, we investigated the extent to which such responses are also present on respiratory muscle nerves of the upper airway. Experiments were conducted on adult cats that had a mictoludic decerebration. The vestibular nerves were electrically stimulated at intensities less than those required to produce current spread to the nearest non-target (i.e., facial) nerve. Vestibular-evoked responses were recorded from the following nerves: recurrent laryngeal, superior laryngeal, hypoglossal, glossopharyngeal, and pharyngeal branch of the vagus nerve. The responses could consist of an increase and/or decrease of nerve discharge. Response latencies (from the 1st stimulus of a 5- shock train) were less than 20 ms; response durations were typically between 12-27 ms. Injections of the neurotoxin kainic acid into the medial and inferior vestibular nucleus abolished the responses. Thus, the widespread presence of vestibular-evoked responses on respiratory muscle nerves of the upper airway, as well as those of the thorax and abdomen, suggests that one function of vestibulo-respiratory reflexes is to provide adjustments in breathing and airflow patency during movements and changes in posture. This does not exclude additional possible roles for vestibular-respiratory reflexes, e.g. in assisting venous return to the heart to counter orthostatic hypotension or in maintaining posture. Supported by NIH grants NS20585 and DC02644.

741.11

Respiratory related evoked potentials (RREP) elicited by inspiratory occlusion were initially recorded from Cz and C4 with a cephalic reference. Subsequent respiratory related evoked potentials have also been reported using either an earlobe or Spinal C7 reference. The difference in the RREP waveform raises the question whether changes in reference site alters the characteristics of the signals recorded. This study compares RREP records referenced to joined earlobe with Cz referenced records. Children between 7-15 years were the subjects. Electrodes were applied to Cz, C4, and referenced to Cz, and joined earlobes. Two trials of 80 inspiratory-inhibited occluded breaths and 1 control trial of 80 unoccluded breaths were presented. Averaged control and occlusion records were compared to determine the presence or absence of the short-latency positive peak (P1). When P1 was observed, the peak latency and amplitude were determined. Analysis of the results showed that with Cz, referencing P1 was observed bilaterally. With joined earlobe referencing P1 was also observed bilaterally. The P1 amplitude with Cz referencing was greater and had a longer latency than with joined earlobe referencing. The observed differences in the RREP P1 peak with the different references can be explained in terms of the increase in noise using a joined earlobe reference. This increases the threshold for resolution of the P1 component from background noise and decreases the amplitude. We conclude that when recording RREP, then consideration must be given to the number of sweeps necessary to maintain a comparable signal to noise ratio between records. (Supported by NIH-NHLBI grant HL 48792).
742.1 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LUTENIZING HORMONE-RELASED HORMONE (LHRH)-ASTROGLIAL INTERACTIONS: EFFECTS OF ESTROGEN DERIVED AND FREE-FLOATING GALANIN PEPTIDES

In recent studies we have provided experimental support for the concept that a significant proportion of GnRH neurons in the median eminence express galanin mRNA and that galanin neurons contribute to the regulation of GnRH release. In the present study, we investigated the effects of estrogen derived and free-floating galanin peptides on GnRH release. The results of this study indicate that galanin modulates GnRH release in a dose-dependent manner. The effects of galanin on GnRH release are suggestive of a physiological role for galanin in the regulation of GnRH release.


The present study investigated the effects of kainate and glutamate on the activity of tanecytes and type II astrocytes in the arcuate nucleus. The results of the study indicate that kainate and glutamate have different effects on the activity of tanecytes and type II astrocytes. The findings of this study are consistent with the hypothesis that kainate and glutamate have different roles in the regulation of food intake.


In previous studies, it has been demonstrated that galanin mRNA is expressed in GnRH neurons. The present study investigated the effects of the LH surge on galanin gene expression in neonatal rat GnRH neurons. The results of the study indicate that the LH surge induces an increase in the expression of galanin mRNA in GnRH neurons.

742.4 GALANIN AND NEUROPEPTIDE Y IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI IN RELATION TO THE ESTROUS CYCLE. T. Alexander, A. Akabas, M. C. Beattie, C. F. Owen, and S. L. Leibowitz.

The present study investigated the effects of the estrous cycle on the expression of galanin and neuropeptide Y (NPY) in hypothalamic nuclei. The results of the study indicate that galanin and NPY are co-expressed in GnRH neurons. The findings of this study are consistent with the hypothesis that galanin and NPY have different roles in the regulation of GnRH release.

742.5 PHENOTYPIC CHARACTERIZATION OF A POPULATION OF PREOPTIC AREA NEURONS ACTIVATED DURING AN LHj SURGE. T. W. Le, S. K. Bellhorn, J. L. Wang, and G. F. Hoffman.

The present study investigated the phenotypic characteristics of GnRH neurons activated during the LH surge. The results of the study indicate that the LH surge activates a subpopulation of GnRH neurons that are characterized by high levels of galanin expression and low levels of NPY expression.

742.6 PHARMACOLOGICAL ANALYSIS OF NEUROPEPTIDE Y (NPY) RECEPTORS MEDIATING NEUROPEPTIDE Y-INDUCED LUTEINIZING HORMONE (LH) RELEASE. D. M. Glasgow, S. Berg, and J. E. Levine.

The present study investigated the pharmacological properties of NPY receptors mediating NPY-induced LH release. The results of the study indicate that NPY activates a subpopulation of NPY receptors that are characterized by high levels of galanin expression and low levels of NPY expression.

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472.7 EFFECTS OF ESTRADIOL (E2) AND PROGESTERONE (P4) ON PROOPOMELANOCORTIN (POMC) AND NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN THE ARCULATE NUCLEUS: A POSSIBLE ROLE IN THE ABILITY OF PROGESTERONE TO SEQUENTIALY ENHANCE AND THEN INHIBIT THE LH SURGE. F.M. Wiesner, A.C., K. Scatchard, 1Department of Physiology, University of Kentucky, Lexington, KY 40504; and 2Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201 and 3Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

POMC and NPY peptides, whose cellular bodies are located in the arcuate nucleus, are thought to play roles in the cyclic release of LH. We tested the hypothesis that changes in the gene expression of these neuropeptides can explain the ability of P4 to enhance the LH surge induced by E2 on the day that it is administered and then to inhibit the surge the following day. Rats were treated with E2 and P4 were implanted with Silastic capsules containing E2. Two days later (day 2), animals received an ip. injection of P4 (1.5 mg/rat) or saline as 1000h. Using this protocol, E2 induced an LH surge during the afternoon of day 2 and P4 enhanced the surge on day 2 and suppresses the surge on day 3. E2-estrogen rats were killed at 0000, 1400, 2300 on day 2 and 0600 and 1400h on day 3. E2-primed rats were killed at 1400, 2300 on day 2 and 0600h and 1400h on day 3. The anterior and posterior arcuate nuclei were dissected, RNA was extracted and POMC and NPY mRNA levels were quantified by solution hybridization RNase protection using 32P-labelled cRNA probes. E2 induced a rhythm in POMC gene expression in the anterior arcuate nucleus; levels reached their nadir at 2300h. P4 prevented this decrease. NPY mRNA levels in the anterior arcuate nucleus appeared to be lowest at 0600h and to increase during the afternoon at levels of 0600h on day 3. Thus, POMC and NPY may play a role in the suppression of LH surges the day after P4 is administered (Supported by NIH AG02234 to PMW).


Glutamate is the most abundant neurotransmitter in the brain and has been implicated in the regulation of gonadotropin-releasing hormone (GnRH) release. The first aim of the present study was to determine if glutamate is contained in axon terminals which could innervate GnRH-neurons in their perikarya or at their nerve terminals in the median eminence. Immunoneurochemical double stainings for GnRH and glutamate show that glutamate is present in numerous punctate juxtapositions to GnRH perikarya as well as to GnRH containing axons in the median eminence suggesting that, from an anatomical point of view, glutamergic neurons can provide axo-axonic and axo-axoplasmatic innervation to GnRH neurons. The effects of glutamate are mediated through activation of 2 families of receptors, the ionotropic and the metabotropic receptors. The second aim of the present study was to determine if GnRH neurons express the kainate receptor and where in the GnRH neurons the receptor protein is located. Immunoneurochemical double stainings for Groth and kainate-2 receptors show that some but not all GnRH perikarya in the median eminence contain immunoreactive kainate-2 receptor protein. Together, the results suggest that glutamergic axons are in an appropriate position to provide excitatory input to the GnRH neuronal system and that some, but probably not all of this input is mediated by activation of kainate-2 receptor subtypes. Supported by NIH HD 24657 (J.L).

472.11 IMMORTALIZED LHRH CELLS (GT1-7) EXPRESS TYPE I PITUITARY ADENYL CYCLASE-ACTIVATING PEPTIDE (PACAP) RECEPTORS; INHIBITION BY PACAP RECEPTORANTIBODIES. B. R. Middeldorp, and C.A. McArdle. Inst. for Hormone & Fertility Research, Inst. of Anatomy, b. Univers, of Hamburg, 22529 Hamburg, Germany; Dept. of Medicine, Univ. of Bristol, Bristol BS2 8BH, U.K.

The release of LH involves signal transduction pathways often linked to the elevation of cyclic nucleotide production. A potent stimulator of cyclic AMP accumulation is the peptide PACAP, whose specific receptor has not been cloned yet. The goals of the present investigation were to determine whether PACAP can activate cAMP production and/or LHRH release from GT1-7 cells, and whether these cells express the type I PACAP receptor. Both PACAP-38 and its truncated form PACAP-27 were potent in elevating cAMP. Similar experiments revealed that these peptides elevated LHRH release 2-3 fold within 10 minutes of treatment, with an EC50 in the low range. Similar results were obtained using PACAP-27. Thus, the PACAP receptor has been described - the short form and five additional forms having 1 to 2 insertions in the third intracellular loop of the PACAP using primers encompassing this region revealed the presence of the short transcript and a single insert transcript in GT1-7 cells. On the basis of these results, we propose that PACAP may be important not only via its actions at the pituitary gonadotrope level but also via the direct regulation of LHRH secretion.

472.8 CENTRAL VIP-ANTISERUM INJECTIONS ALTER THE TIMING OF AN ESTROGEN-INDUCED LH SURGE. L.M. van der Bee, 1J.M.J. Swarts and V.M. Wisse, Dept. Dept. of Neurophysiology, Academic Medical Center, Amsterdam, The Netherlands and Rudolf Magnus Inst Neurosci, Utrecht University, Utrecht, The Netherlands

In the present study, we investigated the effect of intracerebroventricular VIP-antisera (VIP-Ab) injections on the timing and height of an estrogen (E2)-induced LH surge in mature ovariectomized (OVX) female rats. Water rats (n=20) were housed under a 12:12 light:dark cycle and free access to food (5 g/L) and water (20 mL) provided from 9:00 on day 0. The LH surge was induced by two consecutive sc E injections on days 7 and 8. Animals received two saline or VIP-Ab injections (day 8: 22:00 h, day 9: 08:00 h); and hourly blood samples were collected from 8:00 to 18:00 on day 9 and assayed for LH. Two weeks later, rats were used in the second experiment and treatments were reversed (VIP-Ab or saline). During the second experiment, rats were sacrificed between 15:00-16:00 h, and the activation of the GnRH system was evaluated using c-fos immunocytochemistry. VIP-Ab treatment delayed the onset as well as the peak of the LH surge for 1 to 2 h, and significantly reduced the height of the surge. The percentage of c-fos-activated GnRH cells in saline animals (39.8 ± 6.5 %) did not differ from that in VIP-Ab treated females (43.8 ± 8.3 %), and was comparable with that previously found in E2-treated animals. These data demonstrate that blockade of central VIP-activity alters the timing as well as the height of the estrogen-induced surge, but does not affect the activation of the hypothalamic GnRH system.
HYPOTHALAMIC-PITUITARY-GONADAL REGULATION IV

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FOLLISTATIN mRNA IN THE RABBIT BRAIN: IMPLICATIONS FOR A ROLE IN THE REGULATION OF CENTRAL REPRODUCTIVE FUNCTION

D. A. MacCordick, S. Barth and V. J. Roberts. Dept. Repro. Med., UCSD, La Jolla, CA 92037.

Follistatin is a glycosylated single-chain protein originally isolated from porcine follicular fluid. FS binds to the inhibin/activin βA or βB subunits, therefore allowing activin two binding sites for FS and inhibin only one (Nakamura et al., 1991; Endoendorf, 1991). This association is consistent with the apparent specific action of FS on the regulation of activin-mediated effects. The tissue specific differential localization of FS gene expression generally coincides with that of activin subunit expression (Robert and Barth 1994; Endoendorf, 1994). However, in contrast to activin subunit transcripts and mRNAs in the brain, weak FS mRNA signal has only been observed in the olfactory tubercle and layer II of the frontal cortex (Shimanski et al. 1989; Mol. Endoendorf, 1994). We hypothesized that central FS gene expression is more widely distributed and localized in regions coinciding with inhibin/activin β subunits and possibly activin mediated effects.

The present investigation utilized in situ hybridization with a 3P-labeled mRNA splice specific for rabbit follistatin. Abundant FS mRNA is expressed in the olfactory tubercle and layer II and IV of the frontal cortex. In addition, FS mRNA signal was observed in hypothalamic regions where GnRH neurons are localized (nucleus of the diagonal band), in areas associated with the activating pathway (solitary tract nucleus, paraventricular nucleus), and in aspects of the brain with abundant βA immunostaining in cell bodies (striatum, medial vestibular nucleus). These results suggest that FS is localized in sites compatible with a role in the regulation of central reproductive functions.

DEVELOPMENT OF AN OVARINE Y3 cDNA AND EXPRESSION OF THE Y3 RECEPTOR mRNA IN THE OVINE HYPOTHALAMUS AND PITUITARY. 1. M. Matter; and J. D. Young. Department of Animal Science, University of Missouri, Columbia. Animal Physiology Research Unit, Agricultural Research Unit, USDA, Columbia, Missouri 65201.

Neuropeptide Y (NPY) has been shown to exert effects upon the hypothalamic-pituitary-gonadal axis, but the mechanisms through which these effects act are not definitively established. We report herein the development of an ovine cDNA to the putative NPY receptor Y3 (also called LCR1). Ovine hypothalamic mRNA was used in RT-PCR with primers derived from the published bovine Y3 sequence to amplify a 578 base pair (bp) cDNA. Cloning and sequencing revealed a >90% homology to the corresponding bovine sequence. Radiolabeled riboprobes derived from this ovine cDNA were used in a ribonuclease protection assay (RPA) to detect Y3 mRNA in ovine anterior and posterior hypothalamic and pituitary RNA extracts. High levels of Y3 mRNA expression were consistently detected in the pituitary extracts, much lower levels were detected in the posterior hypothalamus (containing the arcuate nucleus). No expression was detected by RPA in the anterior hypothalamus (containing the pre-optic area). The RNA extracts were then subjected to RT-PCR and Northern hybridization. Expression of Y3 mRNA was detected in all samples, demonstrating that low levels of Y3 mRNA, undetectable by RPA, exist within the anterior hypothalamus. These data suggest that the Y3 receptor mRNA is only weakly expressed in the hypothalamus but strongly expressed in the pituitary. Work is now underway to determine if gonadotropins within the pituitary express Y3 mRNA.


The AVPV is a nodal point in neural circuits regulating secretion of gonadotropin and contains sexually dimorphic populations of dopaminergic and dynorphin containing neurons. Despite clearly documented effects of estrogen on TH and D2 receptor expression in the AVPV, little was used on estrogen response elements (ERE). We have identified two ERE-like elements have been not identified in these genes, suggesting that the observed regulatory patterns are from not a direct action of the hormone-bound receptors on estrogen response elements but rather of nuclear factors. For example, the H and PDYN genes contain sequences in their promoters that bind transcription factors thought to play a role in actions of calcium-binding proteins, such as the CaM kinase response element binding protein (CREB). The present histochmical study was undertaken to determine if estrogen alters expression of CREB in the AVP, and possible the estrogen response element binding protein (CREB).

The present histochmical study was undertaken to determine if estrogen alters expression of CREB in the AVP, and possible the estrogen response element binding protein (CREB). 

HORMONAL REGULATION OF PHOSPHORYLATION OF CREB IN THE ANTENTERIOR PERNVERTICAL NASCULUS (AVP). G. Gu, A. A. Rao, M. C. Zee, E. J. Krause and B. Simon. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The antenvernervral nucleus (AVP) of the pituitary is a secondarily sex-dimorphic diencephalic region. It is involved in the regulation of gonadotropin secretion. It contains high densities of receptors for sex steroid hormones and receives strong sexually dimorphic input (SP) containing inputs from the principal bed nucleus of the stria terminalis (BSTp) and posterostral part of the medial nucleus of the amygdala (MeApd). Although both SP, the BSTp and posterostral part of the medial nucleus of the amygdala (MeApd) are involved in the regulation of gonadotropin secretion, cellular levels of SP are not acutely upregulated in the BSTp or MeApd. The present study was undertaken to examine the possible in vivo hormonal regulation of the SP receptor (NK-1R) gene expression in the AVP. Levels of NK-1R mRNA fluctuate during the estrous cycle from a minimum during metestrus to a maximum during proestrus. The correspondence between this regulatory pattern and that of circulating estrogen is supported further by the observation that treatment of ovariectomized rats with estradiol results in a fold increase in NK-1R mRNA in the AVPV. Short term (3 hrs) treatment of estrogen primed ovariectomized rats with progesterone did not alter levels of NK-1R mRNA in the AVP, but after an additional 24 hrs NK-1R levels fell by approximately 40%. These findings suggest that the impact of SP on AVP neurons becomes greater on the day of estrus, when estradiol levels are greatest.

HORMONAL REGULATION OF SUBSTANCE P RECEPTOR (NK-1) mRNA IN THE ANTERIOR PERNVERTICAL NASCULUS OF THE RAT. M.C. Zee, B. Gu, J.E. Krause, and B. Simon. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

NK-1 mRNA levels in the AVPV were measured by in situ hybridization. The mRNA levels were measured in the AVPV of male rats treated with vehicle or 3 mg/100 g estradiol benzoate (EB) for 7 days. Brains were dissected and hybridized with [35S]antisense riboprobes for the mRNA. The probes were initially hybridized to X-ray film and autoradiography emulsion. The signal in supraoptic, paraventricular (PVN) and ventromedial (VMN) nuclei. Quantitative analysis showed a 3-fold increase in neuronal NK-1 mRNA in the VMN of the SP+ rats treated with EB. The increase was restricted to the ventrolateral aspect of the VMN. No significant changes were observed in the hypothalamic PVN. These data suggest that the expression of neuronal NK-1 mRNA in VMN is regulated by estrogen. Moreover, since VMN is an integral part of the neural circuitry controlling lordosis, NO may play a role in female sexual behaviors.

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AGE-RELATED CHANGES IN THE ABILITY OF ESTRADIOL TO INDUCE PROGESTERONE RECEPTOR (PR) mRNA IN FEMALE RATS.


Previous studies have shown that PR mRNA and PR binding increases following administration of estrogen (E). These increases correlate with the ability of progesterone to induce PR mRNA in the medial preoptic area (MPOA), ventromedial hypothalamus (VMH) and arcuate nucleus (ARC). Progesterone-induced PR mRNA labeling was assessed in female rats with a 3P-labeled mRNA probe (sat PR cDNA template kindly provided by Dr. J. Kroll). Female rats of 2.5, 7 and 15 months of age were ovariectomized and one week later injected with 17β-estradiol (1.5 mg in olive oil) or olive oil in the A.M. for three consecutive days prior to sacrifice. Animals were sacrificed in the following eight the following the injection. Estradiol treatment significantly (p<0.01) increased PR mRNA when compared to controls in all three brain regions and at all ages. In the MPOA of 15 month old rats there was a significant (p<0.05) decrease in the ability of E to induce PR mRNA. In the VMH of 15 month old rats there was a significant increase in the ability of E to induce PR mRNA (p<0.01). There was no age related effects in the ARC. However, cohorts of these animals showed no age related deficits in lordosis quotient in response to estrogen and progesterone treatments. These data suggest that specific effects of age on the ability of E to induce PR mRNA. The importance of these changes in the ability of progesterone to induce female reproductive behavior remains to be elucidated.
EXPRESSIO N OF NUCLEAR TRANS-ACTING FACTORS IS REGULATED DIFFERENTIALLY BY OVARIAN STEROIDS IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPV) OF THE RAT. J.W. Pendas, C.A. M. Carr, M.C. Zea, and R.S. Simchoni. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The AVPV represents an attractive model system for identifying cellular factors involved in neuroendocrine regulation of hypothalamic neurons because it plays a critical role in the central control of gonadotropin secretion and contains sexually dimorphic populations of neurons that display distinct patterns of transmitter gene expression. In situ hybridization was used to examine the expression of nuclear trans-acting factors in the AVPV, including the estrogen (ER) and progesterone (PR) receptors as well as the progestronecous, cip, and cip B. Estradiol treatments suppressed expression of cip receptors in the AVPV, and increased levels of PR mRNA were found. Acute treatment of estrogen-primed ovariectomized rats with progestrone (3 h.) increased levels of ER mRNA in AVPV neurons, but did not affect PR mRNA expression, although 27 h. after progesterone treatment PR mRNA appears to decline in the AVPV. ER gene expression fluctuated during the estrous cycle with minimal levels observed on the afternoon of proestrus, and the highest levels during metestrus. In contrast, PR mRNA levels were highest during diestrus and lowest on the morning of proestrus suggesting that PR expression in the AVPV is regulated in a complex manner that does not correspond to circulating ovarian steroid hormones. The expression of cip mRNA in the AVPV also fluctuates over the estrous cycle with maximal levels occurring on the morning of proestrus and with significantly lower levels on the day of metestrus. Preliminary work indicates that acute administration of testosterone induces cip mRNA in the AVPV. In addition, the proestrogenous cip is expressed in the AVPV, but displays no apparent regulation during the estrous cycle, and jun B is undetectable in the AVPV at any point during the cycle.

SEX RESPONSE

743.1


Progesterone (PR) stimulates and advances LH surges in estrogen-primed rats, and pharmacological blockade of P receptors (PR) greatly attenuates spontaneous preovulatory LH surges in proestral rats. Measurements of P during the estrous cycle in ovariectomized rats, however, have revealed that secretion of the steroid occurs prior to the onset of the LH surge. It has therefore been suggested that ligand-independent activation of PRs, which has been demonstrated in vitro (Turgeon & Waring, 1994) may instead function as a component of the surge-generating process. To test this hypothesis in vivo, adult female rats were ovariectomized (OVX) on diestrus 2 and administered an icv injection of estradiol benzoate (EB) sufficient to stimulate LH surges on the following day of proestrus. Additional animals were adrenalectomized (ADX) at the time of OVX, ruling out actions of adrenal P. On proestral proestrus, rats received either RU486 (tmg/kg s.c.) or oil at 12:00h and were killed at 17:00h. RIA of LH in trunk blood revealed that EB stimulated LH surges in OVX and OVX & ADX rats which subsequently received oil. Administration of RU486, however, signals in both groups by 50%. Since endogenous P was eliminated in these animals, our data suggest that RU486 blocks ligand-independent activation of PRs which may normally occur as an important component of the LH surge-generating process. The role of ligand-independent activation of PRs in the generation of LH surges remains to be confirmed by further pharmacological analysis. In current work we are also attempting to characterize the anatomical distribution of hypotalamic satiety and pituitary PRs which may be activated towards this end. (Supported by NIH R01-HD20677, P30-HD28048, P01-HD21921).

743.2


In the ewe, estradiol (E) inhibits luteinizing hormone (LH) pulse frequency during anestra but not the breeding season. Previous studies have demonstrated a role for dopamine in the ewe uterus, which is important in mediation of E action. As a first step in identifying a mechanism for the effect of E on the expression of the immediate early gene product, Fos (a marker of neuronal activation), in cells that also contain ER. Ovariectomized ewes were implanted with estrogen (E) at a slow rate (h=3), or E with estradiol (E) for 48 h. or estrogen and a small dose of progesterone, such as did not induce LH surges. Seven days later, animals were killed for 6 hours at 12 min. intervals and then sacrificed. After fixation, brains were removed, sectioned and stained for ER and Fos using a dual immunofluorescence procedure. E treatment inhibited LH pulses and significantly increased the percentage of ER-containing neurons expressing Fos (2.07% vs. 16.73%) in the preoptic area (POA), but not in other regions of the POA. E had no effect on colocalization of Fos and ER in any other hypothalamic area (AHA, MBH, ARC). E also did not alter the number of neurons containing ER in any area examined. These data suggest that a subset of ER-containing neurons in the POA are involved in the inhibition of E LH pulse frequency by E during anestra. (Supported by NIH HD17864 and HD19668).

743.3

SEX DIFFERENCES IN SERUM LUTEINIZING HORMONE (LH) RESPONSE TO NEGATIVE REINFORCEMENT AND THE EXPRESSION OF CHANGES IN mRNA FOR GONADOTROPIN-RELEASING HORMONE (GnRH) OR THE SECRETAGOGUE INDUCED RELEASE OF GNRH A.A. Bilukati, S.C.H. Hood, and N.B. Schwartz*. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

A continuing puzzle in reproductive endocrinology is the striking sex difference in serum LH response to gonadectomy (gdx), male levels rise within hours, while levels in females do not rise significantly for several days. We have ruled out sex differences at the pituitary level. We measured GnRH transcription and release as a possible source for this sex difference. In situ hybridization was performed using brains from intact, 12, and 24 h post-gdx male and female rats. Serum LH in the males was significantly elevated by 12 h in levels in females had not changed by 24 h. The average number of GnRH mRNA expressing cells, as well as the intensity, rose by 12 h post-gdx in both males and females and remained elevated or began to decline by 24 h. These data demonstrate that the difference in initial rate of serum LH rise in gdx males (rapid) versus females (slow) is not reflected in differences in the early timing of GnRH mRNA synthesis. GnRH release was measured in vitro from perfused median eminences of intact and 24 h post-gdx male and female rats. Tissue was stimulated with NMA, and the resulting GnRH release was measured by RIA. No significant differences were found in GnRH release between groups. This does not rule out sex differences in hypothalamic innervation between the sexes or other regulatory factors. Further research is needed to clarify this issue. Supported by NNSA 1-F32-HD07687 (to AAE), P01 HD-21921 and P30 HD28048.

743.4

CHANGES IN DOPAMINE CONCENTRATION AND ACTIVITY OF HYPOTHALAMIC DOPAMINERGIC NEURONS DURING PREGNACTIN SURGE IN PREGNANT RATS. A.L. Larson, M.E. Herman & M.E. Freeman*. Department of Biological Science, Florida State University, Tallahassee, FL 32319.

The role of tuberoinfundibular dopaminergic (TDIA) neurons originating from the caudal arcuate nucleus (ARN) in modulating prolactin (PRL) secretion is well established. However, the significance of tuberohypophysial DAergic (THDA) neurons and periventricular hypophysial DAergics (PHDA) neurons from rostral ARN and periventricular nucleus (PVN) terminating in the neurointermediate lobe of the pituitary gland in less understood. The aim of this study was to assess the role of THDA and PHDA neurons in PRL secretion by measuring DA concentration and characterizing neuronal activity in ARN and PVN during the diurnal (D) and nocturnal (N) PRL surges of pseudopregnant rats. On the fifth day of pseudopregnancy, brains were harvested after decapitation or fixation at 11:00, 13:00, 15.00, 18:00, 21:00 (D surge) and 23:00, 03:00, 06:00, 09:00 (N surge). PHVns and ARNs of fresh frozen brains were micropunched and DA concentration was measured using HPLC. DAergic neuronal activity was assessed by quantifying double label immunocytochemistry for DA and tyrosine hydroxylase on the fixed brains. Results indicate a decrease in DA concentration accompanied by a decrease in neuronal activity of both PVN and rostral ARN coinciding with the initiation of PRL surges. Middle portions of ARN demonstrated an increase in DA concentration which peaked during the descending phase of PRL secretion. Caudal ARN had a decrease in both DA concentration and neuronal activity during the D PRL peak. These data suggest that changes in DA content of cell populations in PVN and ARN can be attributed to PHDA, THDA and TDIA neuronal activity. Taken together PHDA, THDA and TDIA neurons appear to have differential roles in modulating the dynamics of PRL secretion. Supported by NIH: DK 43200 and HD 11669.
743.5  
**EFFECTS OF CENTRALALLY ADMINISTERED PROLACTIN ON LHRH CONCENTRATION AND RESPONSE TO LHRH IN DOVES.** J. Burdi,* J.P. Atkins, E.J. Rauscher, and W.J. Chaplin. Department of Biological Science, University of South Carolina, Columbia, South Carolina 29208.

The present study was performed to determine the effects of centrally administered prolactin (PRL) on the hypothalamic-pituitary-gonadal (HPG) axis in ring doves (Streptopelia risoria). Doves were injected with PRL (50 or 100 μg/kg) or saline into the lateral ventricle. The concentration of LHRH in the hypothalamus was determined at 0, 5, 10, and 30 minutes after injection. PRL injection resulted in a significant increase in LHRH concentration at 5 minutes, which remained elevated at 10 and 30 minutes. The results suggest that PRL administration may modulate the secretion of LHRH in doves.

743.6  
**EFFECTS OF BICUCULLINE ON THE LH RELEASE IN THE PERIPUBERTAL MALE RAT.** Dai Mitsuhashi* and Fusako Kimura. Department of Physiology, Yokohama City University School of Medicine, Yokohama 222, Japan.

In order to examine the role of γ-aminobutyric acid (GABA) in the control of orcein of puberty in rats, the effects of bicuculline methiodide, an antagonist of GABA receptors, were investigated. Bicuculline methiodide was infused intracranially in peri-pubertal (16-17 days of age), midpubertal (30-31 days of age) and adult (over 45 days of age) male rats for 30 min. Sequential blood samples (110 μl) were obtained every 15 to 30 min from unanesthetized freely moving rats through intracranial cannula and serum LH concentrations were determined by RIA. Although neither bicuculline nor saline infusion significantly altered LH release in peri-pubertal stage, in midpubertal stage, 6 of 8 rats with 20 mg/kg of bicuculline and 5 of 6 rats by 40 mg/kg of bicuculline showed significant response for 15 min at 20 mg/kg and 30 min at 40 mg/kg doses. In adult stage, infusion of both doses of bicuculline induced a prompt increase in LH release in all rats. The significant responses continued for 30 min at 20 mg/kg and 45 min in 40 mg/kg. In addition, direct bicuculline injection into the third ventricle (60 ng/ml) also induced a prompt LH release in adult male rats (p<0.01). In order to examine whether some testicular factors change the GABA receptor mediated inhibition, similar experiments were performed in orchietomized adult rats. Neither 20 mg/kg of bicuculline nor saline infusion induced significant changes in LH release, but 40 mg/kg of bicuculline significantly inhibited the release of LH. It is concluded that, in the male rat, the maturation of the GABA neuronal system to inhibit the release of LH occurs sometime after the onset of puberty and the neurons may partly mediate the control of LH release.
HYPOTHALAMIC-PITUITARY-GONADAL REGULATION

743.11 EFFECTS OF HEMOVARIECTOMY (Hovx) ON OVULATION AND MONOAINE CONCENTRATION IN THE PREDICTANT-ACTING HYPOTHALAMUS (POA-AHA) AND EPIPHYSIS (POA-EPH) OF FEMALE RATS.

It is evident that the mechanisms regulating compensatory ovulation and ovarian response to a present asymmetry and lateralization. Also, that Bovxs modifies the contents of monoamines in POA-AHA. Then, the effects of left or right hemovarectomy (Hovx-L, Hovx-R), performed at 12.00 ± 14.00 h of each day of the estrous cycle on ovulation rate and monoamine concentration in POA-AHA was evaluated. The concentration of norepinephrine and dopamine were not modified by Hovx.

When Hovx was performed on diestrus 2, the ovulation rate was reduced significantly (Hovx-L: 2/6; Hovx-R: 1/6 vs. control 8/8, p < 0.05). The concentration of serotonin in the bovine animal that did not ovulate, presented a different pattern: when the right ovary was extirpated (Hovx-R) the concentration of serotonin was significantly increased (1.524 ± 0.17 ng/mg wet tissue vs. 0.638 ± 0.08, p < 0.001), meanwhile when the left ovary was excised, the serotonin concentration in POA-AHA was similar to control group (0.878 ± 0.26 vs. 0.638 ± 0.08). Present results suggest that the lateralized differences observed on the effects of Hovx on ovulation could be related to changes on the serotonergic levels in POA-AHA.

Supported by DGAPA Grant IN210893, CONACyT IN1719 and PENS

743.12 PLASMA PROGESTERONE LEVELS IN PREPUBERTAL RATS WITH GONADOTROPIN STIMULATION AND CATECHOLAMINERGIC BLOCKADE.

Gonadotropin administration in prepubertal rats increase a induction in follicle growth and steroid secretion mediated by a interrelationship between ovary-hypothalamic-hypophysis axis and diverse systems of neurotransmitters. In this we investigate the participation of the catecholaminergic system on progesterone levels and proioyol follicular growth in 27 days old rats treated with: a) PMSG (8 IU), b) PMSG, 48 h after Re-zepine (RSP, 2.5 mg/kg bw), c) PMSG + RSP, 6 h after HCG (10 IU). Animals were sacrificed 54 and 72 h after PMSG. Results are presented in the next table:

<table>
<thead>
<tr>
<th>Group</th>
<th>Ovulation rate</th>
<th>Ova shed (mg/ml)</th>
<th>Progesterone (nm)</th>
<th>Progestin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td>0/5</td>
<td>0 ± 0</td>
<td>19 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>PMSG+RSP</td>
<td>0/0 ± 0</td>
<td>25 ± 2</td>
<td>5 ± 0.3</td>
<td>a ± 2</td>
</tr>
</tbody>
</table>

All animals treated with HCG ovulated (PMSG+HCG: 6/6; ova shed 28 ± 2; PMSG+RSP+HCG: 6/6, ova shed 17 ± 1). Our results suggest a stimulatory participation of central catecholaminergic system on neuroendocrine regulation of gonadotropin secretion.

Supported by DGAPA, PENS and CONACyT

743.13 BILATERAL ENUCLEATION AT BIRTH DIMINISHES SIMULATED MIDCYCLE GONADOTROPIN SURGES IN ABHEEL FEMALE RATS. T.J. deHaan* and N. Nettlesh. Yale University, Dept. of Ob/Gyn, New Haven CT, 06520.

In rats the secretion of gonadotropins is dependent on an intact suprachiasmatic nucleus. Since (1) the circadian secretion of gonadotropins is sexually dimorphic; (2) estrogen is the developmental hormone that regulates the sexual phenotype of the brain nuclei thus far studied and (3) we have demonstrated immunoreactive estrogen synthetase to be present in axons in the optic tract and in central targets of visual afferents including the suprachiasmatic nucleus, we propose that retinal projections may participate in the development of gonadotropin control.

Experimental: Newborn female rats were enucleated at birth (n=5), left intact (n=5) or sham-operated (n=4). Intact males (n=2) were controls. Three weeks later all animals were castrated. After 3 weeks a stimulated midcycle surge was induced by two daily subcutaneous estradiol injections (10 μg/kg) plus 500 μg progesterone s.c. 24 h later. Results: The area of LH surge in intact females was similar to control group (0.278 ± 0.26 vs. 0.638 ± 0.08). This suggests that enucleation eliminates or blocks the midcycle surge. In addition to supporting our hypothesis of a developmental role of the visual tract via estrogen formation, these studies indicate that this process may be underway prior to birth. (Supported NIH Grant HD15857 to F.N. Brown-Coxe Fellowship to T.J.H)

743.15 ESTRADIOL-17β ACUTELY INCREASES POMC mRNA LEVELS AND ESTRUS-DEPENDENT SECRETION FROM THE RAT SCROTAL HYPOTHALAMUS.
A. Day, N. Bosvesv,
Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6505; Dept. of Animal Sciences, Rutgers University, New Brunswick, NJ 08903; Mt. Sinai Medical Center, New York, NY 10029-6574.

The role of estrogen in the regulation of hypothalamic-estradiol neuronal activity was determined in vitro and in vivo. Using primary cultures of cells from fetal mediobasal hypothalam, we determined the effect of estrogen (estradiol-17β) on B-EP precursor POMC mRNA levels and the B-EP secretion in vitro. Estradiol-17β dose-dependently increased the levels of B-EP from the cultured neurons between 3-24 h. However, these cultured neurons developed desensitization to the stimulatory effect of estrogen at 48 h. The steroid also increased cellular levels of POMC mRNA at 24 h in vivo. 24 h following s.c. injection of 10 μg estradiol benzoate, ovxeanestrous rats showed a significant increase in B-EP concentration in corpora lutea and with those in oil-treated controls. These data suggest that, in addition to known inhibitory effect of estrogen, the steroid may also stimulate B-EP synthesis and secretion. Supported by National Institutes of Health Grants AA87857 and HD20498.

743.16 EVIDENCE THAT PROGESTERONE INCREASES THE NUMBER OF DELTA OPIOID RECEPTORS IN THE PREOPTIC HYPOTHALAMUS OF THE EWE DURING THE LUTEAL PHASE OF THE ESTRUS CYCLE.
L. Clark*, B. Thom and C. Caspy.
Prince Henry's Institute, P.O. Box 5152, Clayton, 3168, Australia.

The extent to which endogenous opioid peptides regulate GABAergic activity may vary with physiological status. We have measured the number and affinity of [3H]U69593 binding to the delta opioid receptor in the preoptic area (POA) of the hypothalamus of groups (n=4) of ovariectomized (OVX) ewes that were either untreated or given estrogen (E2), progesterone (P), or E and P for 10 days. We also studied ewes (6-kg/group) at various stages of the estrous cycle. POA membranes were used for Scatchard analysis for the 5 subtypes of opioid receptor (Then et al., 1995. J. Endo. Int. Press).

In OVX ewes, P increased mean (±SEM) δ opioid receptor number (27.82±4 vs 39.19 ± 3.8 density of binding sites in control) when compared to normal in affinity and E had no effect on other receptor subtypes. In cycling ewes there were more δ receptors in the luteal phase of the cycle (33.1 ± 1.6 fmol/mg protein) than in the early (41 ± 12 fmol/mg), mid- (68.12 ± 9.23 fmol/mg) or late- (35.81 ± 5.4 fmol/mg) follicular phase. The number and affinity of the μ and κ receptors was similar across the cycle.

The data suggest that the δ subtype of opioid receptors in the POA is regulated by P and the higher number in the luteal phase of the cycle could account for increased opioid tone at this time.
IS A DECREASE IN OPIOID TONE NECESSARY FOR THE TIMING OF THE LH SURGE IN RATS? P.B. Lieberman, C.A. Tannenbaum, J. J. Zhang, E.A. Schwartz, A.J. Forman, Department of Physiology, Pharmacology, and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

HYPOTHALAMIC-PITUITARY-GONADAL rats related data was of CONSISTENT vehicle hypothesized. Based on the observed changes in LH secretion following removal of the anterior hypothalamus in rats, it was hypothesized that there is a critical period during which LH secretion is controlled by the anterior hypothalamus. This critical period is characterized by a surge in LH secretion that is associated with the onset of estrus. The LH surge is regulated by the secretion of gonadotropin-releasing hormone (GnRH), which is produced in the hypothalamus and acts on the pituitary gland to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).


In addition to hypothalamic GnRH, local inhibitory factors (e.g., BDNF) play a role in regulating the GnRH neurons. These factors act through a negative feedback mechanism to inhibit GnRH secretion. The study found that the removal of local inhibitory factors led to an increase in LH and FSH secretion, suggesting that these factors play a role in modulating GnRH neuron activity.

743.20 EFFECT OF FROGSTERONE ON LHRH, LHRH RECEPTOR AND LH mRNA LEVELS IN OVARIOTOMIZED AND ESTROGEN-TREATED RATS. D. E. colleagues. The study investigated the direct and indirect effects of LHRH and LH mRNA levels in response to estrogen treatment and the potential role of LHRH receptor density in regulating LH secretion.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION VI

744.1 INFLUENCE OF LIFE-LONG MODERATE CALORIC RESTRICTION ON NEUROPEPTIDE Y EXPRESSION IN THE ARCULATE NUCLEUS OF FEMALE RATS. T. M. McNeary and P. M. Wise, Department of Physiology, University of Kentucky, College of Medicine, Lexington, KY 40536-0684.

Moderate caloric restriction extends lifespan, reduces the incidence of age-related diseases, and delays reproductive senescence in rats. We have previously shown that life-long moderate caloric restriction delays onset of proestrus and reduces LH and testosterone pulses in female rats. Neuropeptide Y (NPY) is thought to play an important role in maintenance of reproductive cycles, feeding behavior, and metabolic homeostasis. We hypothesized that moderate caloric restriction would result in changes in NPY expression in the arcuate nucleus, a key area regulating reproductive aging. Female Sprague-Dawley rats (7 weeks old) were placed on caloric restriction (CR) or control (C) diet at 7 weeks of age. NPY immunoreactivity was assessed at 14, 120, and 360 days of age using immunohistochemistry.

744.2 SODIUM PENTOBARBITONE AND THE INHIBITION OF LUTEINIZING HORMONE PULSES IN RATS: THE SIGNIFICANCE OF HYPOTHERMIA. P. H. Strawson and C. W. Cong. Division of Biomedical Sciences, King’s College, London WC2R 2LS, U.K.

The role of hypothermia in the suppression of pulsatile luteinizing hormone (LH) release by the general anaesthetic sodium pentobarbitone has been investigated in ovariectomised rats. Each animal was fitted with an intraperitoneal miniature radio transmitter to monitor core temperature with an indwelling cannula in the right atrium of the heart and in the intraperitoneal space. During the 6-hour sampling period the animal’s core temperature was recorded automatically every 3 minutes and a 25 ml blood sample was taken at significant effect on LH secretion. After the initial 3 hours of sampling either the drug or the vehicle was administered via the intraperitoneal cannula from outside the cage ensuring minimal disturbance to the animal. Administration of pentobarbitone at an ambient body temperature of 21°C resulted in a significant hypothermia throughout the 3-hour post-injection period. During this period there was a significant reduction in mean LH concentration, pulse amplitude and duration. The effect of pentobarbitone on LH secretion was studied at an ambient body temperature of 35°C and there was no significant reduction in core temperature and no significant change in the LH pulse parameters. Administration of pentobarbitone under these conditions resulted in an increase in the LH pulse parameters at either 21°C or 35°C. These results indicate that the effect of this barbiturate on the pulsatile release of LH is secondary to the induced hypothermia. We are investigating the extent to which the induction of hypothermia may be critical importance in the suppression of LH pulses by other drugs.
744.3 VOLUNTARY EXERCISE INHIBITS BASAL GONADOTROPIN SECRETION IN OVARIECTOMIZED, NON-REPLACED, BUT NOT OVARIECTOMIZED, GONADOTROPIN-RELEASING HORMONE TRILDEGLUTETRIDE-SUBSTITUTED HORMONE TREATED ESTRADIOL-treated, David Pinger, Haythem Ali, Subhashini Ladella, Catherine Loboki, and Karatna Borer. Providence Hospital, Department of Physiology, Southfield, MI, and the University of Michigan, Department of Kinesiology, Ann Arbor, Michigan.

Recent studies in male hamsters have shown that voluntary exercise facilitates GnRH-mediated secretion by inhibiting the negative feedback of testosterone. The present study investigated the influence of exercise on basal (morning) gonadotropin levels in ovarioctomized hamsters treated with blinded or capsulated estradiol.

Forty-eight adult female hamsters were ovarioctomized and implanted with capsules which were either blank or contained 4 mm of estradiol 17β (ED). Two weeks after orchex CT group was sacrificed, in cages with wheels (EX group) or in similar cages without wheels (SED). Blood samples were obtained between 8 and 10 AM by retro-orbital puncture before surgery and at weekly intervals following surgery for 5 weeks.

There was a dramatic increase in serum LH and FSH following ovarioctomy in both groups without ED, but all ED-treated hamsters had low levels throughout the study. There was no consistent difference in LH or FSH levels between the TED and ED groups with ED, but the serum FSH (and to a lesser extent LH) was consistently significantly lower in the ED than TED groups with blank capsules.

We conclude that exercise has a steroid independent effect to inhibit basal gonadotropin secretion in female hamsters.

744.5 SUPPRESSION OF LH PulseS BY REDUCING GLUCOSE Availability IS MEDIATED BY SENSORS IN THE LOWER BRAIN STEM. S. Naganta1, K. Murakami1, D. C. Bucella2, D. C. Trabulski1, M. A. C. Fosco1, H. Trakalam2, D. Eisinger1 and K. L. Mace1. 1Dept. of Biological Sci., Nagoya Univ., Nagoya 464-851, Japan; 2Regen. Sci. Prog. Univ., Michigan Ann Arbor 48109, USA.

Glucose may play a key role in controlling reproductive activity through modulation of GnRH secretion. The present study in the rat determined 1) if glucopenia of suprasellar LH release is sexually differentiated, and 2) if the glucopenic effect is potentiated by gonadal steroids. 3) if such glucopenic suppression of LH secretion is mediated by a glucopenic effect on the pituitary. Our approach was to monitor pulsatile LH secretion after peripheral (pugal) or central (4th ventricle, 4V) administration of the competitive inhibitor of glycogenolysis, 2-deoxyglucose (2DG). Four days after gonadectomy, blood samples for LH were collected every 6 min for 3 h. After one hour of sampling, ZDG was administered peripherally (200, 200 or 260 mg/kg) in gonadectomized (GDX) males and females in the presence or absence of sex steroids (testosterone or estradiol). LH was collected and tested centrally or peripherally (40 or 40 mg/kg) in GDX testosterone-treated (T) males. In the presence of steroids, mean plasma LH decreased (p < 0.05) at all peripheral doses of 2DG, whereas in the absence of sex steroids, the lowest dose was ineffective. The middle dose of 2DG suppressed (p < 0.05) mean plasma LH in GDX females but not in GDX males. Central 2DG infusion of 40 mg/kg, but not 4 mg/kg, suppressed pulsatile LH secretion in both GDX and testosterone-treated GDX males. LH secretion was not depressed by an isosmotic xylose infusion. The site-specificity of the 2DG infusion into the 4V was confirmed by an isovolumetric infusion of dye at the termination of sampling. Collectively, our findings suggest that (1) the hypothalmo-hypophyseal axis of female rats is more sensitive to the decreased glucose availability induced by 2DG than that of males; (2) this glucopenic suppression of LH pulses is potentiated by gonadal steroids; (3) glucose availability is detected in the 4th ventricle consistent with the notion that the area postrema may serve as an important glucosensor.

744.6 LUTEINIZING HORMONE SECRETION IN RHEUS MONKEYS FOLLOWING CORTISOL WITH SYNTHETIC METYраОRINE (CRH) AND METYраОRINE (CRH) AND MetIrine (CRH), and various stressors which increase CRH secretion cause inhibition of LH secretion. In an attempt to further elucidate the physiological significance of CRH regulation of LH secretion we determined the effects of metyrapone on LH secretion. Metyrapone inhibits the synthesis of CRH neurons, presumably as a consequence of reduced cortisol negative feedback. We measured that pituitary CRH content and plasma CRH level in response to metyrapone (3 mg/kg/hr) or saline was infused for ten hours from 2300 to 0900 hours. Blood samples for LH and cortisol were collected at 15 minute intervals for six hours starting at 0900 hours. Metyrapone infusion resulted in a pronounced inhibition of cortisol. The mean (± SEM) cortisol level at the end of a 10 hour infusion of metyrapone was 6.7 ± 0.6 μg/dL compared to 46.3 ± 3.9 μg/dL in saline controls. Cortisol levels gradually increased to 22.2 ± 3.5 μg/dL during the subsequent six hour blood sampling protocol compared to 53.1 ± 5.4 μg/dL in the control group. Mean LH levels were 73.4 ± 5.0 μg/dL following metyrapone compared to 75.5 ± 6.1 μg/dL in saline controls. No effect of metyrapone infusion on pulsatile LH secretion was seen. These results indicate that inhibition of LH secretion does not always accompany activation of CRH neurons. The presence of CRH populations in the hypothalamus that regulate the pituitary-adrenocortical axis specifically may explain these findings. Alternatively, CRH may not be a critical neuromodulator of LH secretion in the ovarioctomized monkey (This work was supported by the MRC of Canada).
HYPOTHALAMIC-PITUITARY-GONADAL REGULATION VI

744.9 CROSS-CORRELATION ANALYSIS OF INTERACTIONS BETWEEN HYPOTHALAMIC UNITS ASSOCIATED WITH THE GONADOTROPINS-RELEASING HORMONE (GnRH) PULSE GENERATOR. T. Ong and E. Keizer. Laboratory for Neuroendocrinology, Medical School, The University of Texas Health Science Center, Houston, TX 77225.

The electrophysiological correlates of the activity of the GnRH pulse generator, a hypothalamic oscillator that governs the episodic secretion of the pituitary gonadotropins, are studied in vitro in excitable neurons (MUA volleys) of the medio-mesial hypothalamus (MMH) that represent the simultaneous increase in firing rate of individual units. This synchrony could be effected by parallel inputs from a "master oscillator" or by being identified by clustere analysis of multunit recordings from the MMH of 3 ovariolectomized monkeys and their connectivity analyzed by cross-correlation histograms of time series of their action potentials, a method that the firing of two neurons registered to one another is not due to chance. To relate the results to the generation of the MUA volleys, the analyses were performed separately during the volleys and the intervals between them. Significant (p<0.01) interaction was found in >60% of the 31 pairs of pulse-generator-associated units analyzed. The majority of cross-correlation histograms consisted of a single peak with a delay time of zero-3 ms and a duration of 0.5-1 ms between and during volleys. The distinct character and duration of this peak suggest, in agreement with observations made by others in the cerebral cortex, direct, synaptic excitatory interactions between these units. Although the specific characteristics of these interactions (strength and contribution) varied considerably from one unit pair to another, they were not significantly different during and between volleys. The results suggest that the synchronization of the single-unit burst may be effected by these direct synaptic interactions rather than by a common Zeitgeber. (Supported by NIH grants HD-17438 and HD-08610, and by the Elwood Foundation.)

744.10 MODELING THE AGONIST-INDUCED ELECTRICAL AND CALCIUM ACTIVITY PATTERNS OF PITUITARY GONADOTROPS. Y.-X. Li, S.-S. Stoica, and S. Keizer. Department of General Medicine, Tokyo University, Tokyo 113, Japan.

Bacterial endotoxin lipopolysaccharide (LPS) can induce a variety of immune responses including a release of tumor necrosis factor (TNF)-α into peripheral circulation and suppress gonadotropin secretion as well. We examined in the rat whether LPS could affect the electrical activity of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator, which governs pulsatile secretion of gonadotropins from the pituitary, and the involvement of TNF-α, if any, in this process. Ovariectomized rats were fitted with chronically implanted electrodes to record electrical and intracellular activity of GnRH neurons. The rats were injected intravenously with LPS (1 μg/kg, MUA volleys and associated luteinizing hormone (LH) pulses were suppressed for several hours. The suppressive effect of LPS was nullified by the antibody against TNF-α administered intracerebroventricularly (ICV). Graded doses of TNF-α administered either IV (0.4, 1 and 2 μg/kg) or ICV (20, 50, and 250 ng) suppressed both MUA volleys and LH pulses in a dose-dependent manner similarly as LPS did. On the other hand, neither IV nor ICV injection of TNF-α affected MUA volleys if animals were pretreated with indomethacin (1 μg/kg), a cyclooxygenase inhibitor, 10 min before. These results suggest that LPS leads to a decrease in hypothalamic GnRH pulse generator activity through TNF-α, and that the suppressive effect of either peripheral or centrally derived TNF-α on the pulse generator depends on the proinflammatory cytokines.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995


The secretion of LHRR has a distinctive, pulsatile pattern of short periods of relatively high levels of release followed by longer intervals of low levels of release. A simple model consisting of 100 randomly connected LHRR neurones. All neurones projected to the median eminence, were endogenous bursters with randomly assigned inter-burst intervals (IBI), and had excitatory effects on postsynaptic cells. Each neurone multiplied its inputs by the associated synaptic weights and sums these values. Neurone output was a sigmoid function of the sum and circuit output was the summed neurone output. This process was repeated every sec for 4-8 simulated hours. With 0% connectivity, circuits had only small fluctuations in output over time. A small cell set of 20 (i.e. 3% of the entire pool) was shown to produce a repetitive rhythmid, synchronous pattern of electrical activity from the entire circuit. Ablation of the LHRR neurones appeared to fail to simulate a spontaneous pattern of electrical activity from the circuit. The model has numerous variables that require further evaluation but appears to be a useful paradigm for modeling LHRR pulsatility.


Control of the female HPG axis by estradiol (E2) involves inhibition of hypothalamic GnRHR release via modulation of the β-endorphin system. μ-opioid peptides (e.g. β-endorphin) hyperpolarize hypothalamic cells by opening a K+ inward-rectifier (I_{Ks}). Intracellular recordings in arcuate neurones from in vitro hypothalamic slices from ovariectomized guinea pigs were used to study the mechanism of E2 action. The potency of the response to the μ-opioid agonist, DAMGO, is rapidly decreased nearly four-fold by a brief exposure to E2 (100 nM, 20 min) in about 35% of neurones tested. The effect of E2 is concentration-dependent. The receptor/G-protein coupling is altered by cAMP-dependent protein kinase (PKA) and there is evidence that the estrogen receptor interacts with PKA in some systems. The effects of E2 were blocked by the nonspecific protein kinase inhibitor, staurosporine (100 μM, n=10), and mimicked by stimulation of adenylate cyclase with forskolin (1-20 μM, n=6). The more selective PKA activator, Sf-cAMP mimicked the effects of E2. The present work provides a novel transduction mechanism for E2, i.e. PKA activation, as well as elucidating the intracellular mechanisms regulating the actions of opioid peptides. (Supported by PHS Grants DA05158 and MH10327)


We have previously shown a rapid effect of 17β-Estradiol (E2) to alter the potency of the μ-opioid agonist, DAMGO, in hypothalamic neurones. This effect is faster than would be expected for the classic genomic model of E2 action, and appears to involve activation of PKA. To study the mechanism of this rapid, intracellular changes were made from control neurones to in vitro hypothalamic slices from ovariectomized guinea pigs. DAMGO hyperpolarizes these cells by opening an inwardly rectifying K+ channel. The potency of this effect is reduced nearly four-fold by a brief exposure to E2 (100 nM, 20 min) in about 35% of neurones tested. The EC50 for E2's effect was 8 μM, with a Hill slope of 0.7. The biologically inactive isomer, 17α-E2, was unable to mimic the effects of 17β-E2. The effects of E2 appear to be mediated by an intracellular receptor, as this membrane-impermeant conjugate BSA-E2 did not alter DAMGO potency. Furthermore, the effects of E2 were blocked by the "pure" antiestrogen ICI 164,384 and the nonsteroidal estrogenic drug, diethylstilbestrol. By using an antagonist to shift the E2 concentration-response curve, Schild analysis resulted in an estimated KI for ICI 164, 384 of 0.3 μM, similar to the K+ for ICI 164, 384 binding to classical estrogen receptor. Therefore, the characteristics of the receptor mediating this rapid estrogen effect are in many ways similar to the classical estrogen receptor and this model may play a novel signal transduction mechanism for this protein. (Supported by PHS Grants DA05158 & MH10327)
745.1

EFFECTS OF MICROPLANTS OF AN ANTIANADROGEN INTO THE ROSTRAL HYPOTHALAMUS ON GABAergic NEURONS AND ON LH SECRETION IN THE INTACT MALE RAT. D.R. Grauman, M.S. Rosen, M.M. McCarthy and M. Selman. Center for Studies in Reproduction, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

GABAergic neurons in the rostral hypothalamus are stimulated by testosterone. To investigate whether this action is mediated locally through androgen receptors in the rostral hypothalamus, bilateral microplants (28 gauge) containing the antianadrogen flutamide, hydroxyflutamide, or vehicle, were stereotaxically implanted into the rostral preoptic area just dorsal to the major population of GnRH cell bodies. Two days later, blood samples were collected for LH assay and animals were sacrificed by transcardial perfusion. The rostral GnRH-ergic neuronal activity in tissue microdissected from the site of the implanted cannules. Animals were decapitated either without treatment, or 60 minutes after injection of GnRH degradation by AOA (0.1 units/ml), and the rate of GnRH accumulation in the tissue after AOA administration is a measure of a GnRH turnover. Levels of mRNA for both forms of glutamic acid decarboxylase (GAD65, GAD67, the rate limiting enzyme responsible for GABA synthesis, and also measured by reverse microcannula injection of testosterone propionate in each male rat. (Supported by NIH grant HD25151 awarded to MS).

745.2

TESTOSTERONE-INDUCED ACTIVATION OF TYROSINE HYDROXYLASE-CONTAINING NEURONS OF THE A14 AND A15 HYPOTHALAMIC NUCLEI IN THE MALE SHEEP. L. Bovenkerk, J.E. Hekman, E.E. Hettema, J.C. Bovenkerk, and J. Jackson. 3rd Dept. of Veterinary Biosciences, University of Illinois, Urbana-Champaign, IL 61801, Dept. of Anatomy and Cell Biology, University of Cincinnati, Medical Center, Cincinnati, OH 45267.

Tyrosine hydroxylase (TH)-containing neurons of A14 and A15 hypothalamic nuclei, which are dopaminergic in nature, appear to mediate the inhibitory effects of gonadal steroids on luteinizing hormone releasing hormone (LH) secretion in the male sheep. However, the role of these neurons in steroid-induced inhibition of LH release in the male is unknown. We tested the hypothesis that in the male TH neurons of the A14 and A15 are activated by testosterone propionate (TP) in the rostral hypothalamus. We examined the period of the TH response to express cFos, a marker of neuronal activation, in castrated male sheep infused with vehicle (n=4) or TP (766 pg/kg/d; n=4) for 72 h. Blood samples were collected every 10 min for 4 hr prior to and during the final 4 h of infusion. Animals that were euthanized and hypothalami were collected. Coronal sections (16 pm) were cut through the A14 and A15 nuclei and a series of every 16th section was assayed for TH and cFos colocalization using a dual-immunoperoxidase procedure. The infusion increased circulating T (p<0.01), decreased mean LH (p<0.01) and increased LH interburst intervals (p<0.01). T infusion increased the percent of TH cells expressing cFos in the A14 (n=0.01; 40.3%) (80/280 TH cells) vs. 21.15% (77/371 TH cells) and in the A15 (n=0.01; 33.7% (206/621 TH cells) vs. 7.92% (579/747 TH cells). These results show TH-containing neurons of the A14 and A15 are activated by T infusion and suggest they may be involved in steroid-induced inhibition of LH release in the male. (Supported by AG-27302-8177).

745.3

NONGENOMIC TEMPORAL ACTION OF THE NEUROENDOCRINE STEROID, 3a-HYDROXY-4-PREGNEN-20 ONE (3aHP) IN GnRH-INDUCED SUPPRESSION OF FSH IN PERITUBULAR PITUITARY CELLS. J.E. Wiswall and M. Wolfe. Hormonal Regulatory Mechanisms, B&G Building, Univ. of Western Ontario, London, Canada N6A 5B7.

The recently discovered neurosteroid 3aHP, has been shown to selectively suppress GnRH-induced pituitary FSH release by actions at the level of the gonadotropic membrane Ca2+ channel and the cell signaling pathway involving protein kinase C and CAMP production (Endocrine-Regulation 1991, 89: 134-371; 1994, 134-377). To determine the time course of action of 3aHP in FSH suppression, pituitary cells from random cycling female Sprague-Dawley rats were allowed to attach to coated dishes (4 days), transfected with glial fibrillary acidic protein 1 and for 10-min samples collected for 30 min. Cells were incubated 8 min then 10-min samples collected for 30 min. Cells received a 5 min pulse of GnRH (10 −6) at 30 min (Tr; control) and at 270 min (Tr; treatment); Tr-0 = start of pulse, Tr-5 = end of pulse. Microplants of GnRH and/or 3aHP were applied to the sectioned tissue used in combination with the Tr pulse. FSH was determined in all samples by specific RIA. GnRH stimulated (n=5) at 60 min after start of pulse in a significant (0.5-3.5 fold peak) of FSH release. Later, GnRH-stimulated FSH release was 0.135±0.02, 50-100% of the control, was applied at Tr-0, the GnRH-induced FSH release was completely suppressed. A 3aHP pulse at Tr-0, Tr-3, Tr-5, Tr-7, Tr-10, and Tr-13, the GnRH-induced FSH release was 0.135±0.02, 50-70%, 100-105%, and 105%, respectively, of the Tr-0 peak. A 5 min pulse of MHP, prostaglandin, or estradiol at Tr-0 resulted in 105, 155, and 245%, respectively, of the Tr-0 FSH peak. Pretreatment of cells with estradiol prevented the suppressive action of 3aHP. This result indicates that 3aHP most effectively suppresses GnRH-induced FSH release when present at the start of GnRH release and in the absence of estradiol. They suggest that 3aHP action may be at the level of the GnRH-receptor binding. (Supported by NSERC of Canada).
HYPOTHALAMIC-PIITUITARY-GONADAL REGULATION VI.

475.7


The lateral septum (LS) relays hippocampal input to a variety of hypothalamic areas modulating the neuroendocrine output. Especially, the LS has long been known to be involved in reproductive processes. In line with this and using a tract-tracing technique, we investigated the existence of a direct projection from the LS neurons to the gonadotrophin (GnRH) efferent neurons.

Nine adult male Wistar rats were used. A unilateral microinjection of a biotinylated-dextran into the ventral LS and anterograde transport was allowed to proceed for 15 days. The brains were then perfused with PBS containing 4% paraformaldehyde and biotin and cut on a freezing microtome. All sections were double-stained to reveal the LS projection fibers with the avidin-peroxidase-diaminobenzidine technique and the GnRH neurons using the ABC method. Appositions between LS efferents and GnRH-immunoreactive (ir) neurons were located at the light microscopic level.

In all cases such appositions suggestive of potential contacts were found to involve 20-30% of the GnRH-ir neurons located ipsilaterally to the injection site. There was no obvious dorso-ventral or antero-posterior topographic arrangement of the contacted neurons that were found in the preoptic region as well as in the suprachiasmatic hypothalamic continuum. Potential contacts between labelled LS efferents and GnRH-ir neurons were observed on the proximal dendrites as well as on the perikaryon of the GnRH-ir neurons. The majority (80%) of the contacted GnRH-ir neurons were of the irregular sub-type. The synaptic nature of these appositions between LS efferents and GnRH neurons is presently under study at the ultrastructural level.

These results suggest the existence of a direct, monosynaptic projection from the LS to a sizeable component of the GnRHergic neuroendocrine apparatus. The nature of the LS information relayed to the GnRH apparatus remains to be clarified.

Supported by INSERM.

475.9

POST-TRANSCRIPTINAL REGULATION OF THE GONADOTROPIN-RELEASING HORMONE (GnRH) GENE IN GTI-7 CELLS. ALFRED W. WRAY, PHD, M. J. ROBERTS, PHD, C. FISCHBERG, MD, M. J. ROBERTS, Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, New York, NY 10029.

Several groups have reported that treatment of GTI-7 cells with phorbol myristate acetate (PMA), a phorbol ester, caused a decrease in the cytoplasmic GnRH mRNA concentration. While there is an inhibitory transcriptional effect of PMA, there is also the possibility that this may be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA, but there appears to be an effect on enhancing GnRH mRNA degradation.

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Cellular Analysis of Tyrosine Hydroxylase Expression in Lactating Rats

745.1

INTERHEMISPHERIC ASYMMETRIES IN 2-DEOXYGLUCOSE UPTAKE AND OPEN-FIELD BEHAVIOR OF CALLOSAL AND ACALLOSAI MICE. E. R. Schmitz*, D. P. Weller, and H. L. Pape. Institute of Anatomy, Univ. of Zurich, Inst. of Anatomy, Univ. of Lausanne, Switzerland.

In order to study functional lateralization in mice, we measured the uptake of 2-deoxyglucose (2DGU) in the left and right side of the brain of mice while they were freely moving in an open-field. Ten C57BL/6J mice and 12 sex- and age-matched mice with callosal agenesis were injected i.p. with a solution of [1-14C]-2-Deoxy-D-Glucose (2DG), and immediately released in a dimly illuminated open-field area (150 cm). Their paths were recorded and analysed off-line to assess activity, turning preferences and distance to the wall as a measure for exploration versus anxiety. After 45 min., the mice were sacrificed, and the brains processed for autoradiography. The amount of 2DGU in genetically defined regions (subcortical and 11 cortical) was assessed by means of a computer-aided image analysis. Acallosal mice showed a high propensity for circling, the degree of turning preferences to the left or to the right being significantly correlated with 2DGU-asymmetries in the substantia nigra and the diagonal band of Broca. These correlations were missing in the callosal C57Bl mouse. In both groups of mice, the 2DGU asymmetry in the lateral amygdala was correlated with thigmotaxis, animals with high activity in the right amygdala spending most time in the center of the open-field. We conclude that the presence of a corpus callosum helps to correct asymmetrical influences from ascending activation systems on locomotor directionality. Independent of the corpus callosum, there appears to be functional lateralization of fear-related processes in the amygdala. Supported by Swiss National Foundation 31-37497.

745.2


The anterior ectosylvian cortex of the cat receives different sensory inputs and has been considered an area for sensor-motor integration. We recorded somatic responses in neurons along the banks of the anterior ectosylvian sulcus of an alert cat and investigated possible motor correlates by electrically micro-stimulating (~40 μA) recording electrodes fixed in the head-fixed condition, stimulation evoked eye movements that were driven to a fixed contralateral orbital position. When the head was fixed, with body free, the same stimulus drove gaze (eye-head) shifts towards a fixed contra-position relative to the body: both the amplitude and direction of evoked gaze movements were modulated by initial gaze position. When the body was free, the same stimulus drove coordinated rapid gaze and contralateral reaching forelimb (paw) movements, and body postural adjustments. Gaze looked at, and paw went to, a fixed contra-spatial location relative to the initial body axis. For movements beginning with gaze, head and body aligned, the location of visual receptive fields and the preferred direction of moving visual targets were correlated with the final position of evoked gaze shifts and paw movements. The cortical area which evoked eye, head and forelimb movements was located mainly in the middle one-third of the ventral bank and the fundus slightly extending to the dorsal bank. A topographic representation of 3-D space re-body may exist in this cortical area. We do not know whether stimulation generated a 'target' that the animal tried to 'catch' or an eye-head-limb motor program.

745.3


We have studied activity patterns of mastication-related neurons (MRN) in the orofacial first somatosensory cortex (SI) of conscious cats. Over 90% of MRNs in the orofacial SI showed activity patterns that followed the masticatory rhythm (RB-MRN). About 20% and 40% of RB-MRN received inputs from the tongue and perioral regions. The RB-MRN with the receptive fields in the tongue (T-RB-MRN) and perioral regions (P-RB-MRN) showed the regular burst firing corresponding to the jaw-opening to -opened and the jaw-closing to -closed phase, respectively. But they showed the irregular one after food intake. T-RB-MRN showed also firing frequency changes in burst firing that were dependent on differences in food texture, but P-RB-MRN did not. Furthermore, to investigate the function of T- and P-RB-MRN during mastication, we examined the activity of each lemur in the perioral or tongue projection area in SI on masticatory behavior. Lesions were created by injection of kainic acid (1%, 2μl). After 24 hr of healing, we observed the behavioral deficit of oral cortical behavior during mastication. T- and P-L animals showed the progression of the masticatory process. On the basis of these findings, we will discuss the functional sensory information in the orofacial SI for the performance of mastication.

745.4


The present study was designed to examine the basic characteristics of facial nerve-evoked neural activities in the primary somatosensory (SI) and motor cortex (MI). Under anesthesia, adult rats were implanted with a stimulating electrode on the buccal branch of the left facial nerve. Field potentials (FP) and multiple unit activity (MUA) evoked by facial nerve stimulation were recorded. Facial nerve stimulus elicited primarily positive- or negative-positive potentials in layers II-VI bilaterally in SI and Ml. Large negative latencies were always accompanied by a salient MUA indicating a synchronous excitation of local neurons. The primary response latency was 4.9 ms for contralateral SI, 9-10 ms for contralateral MI and 13-15 ms for ipsilateral SI. Current source density (CSD) analyses of laminar FP profiles in contralateral SI revealed two primary sinks at 400-500 μm (E1a) and 1000-1200 μm (E1b) from the cortical surface. E1a and E1b were followed by a secondary sink in superficial 200-600 μm, E2a and deeper layers (1200-1400 μm, E2b), respectively. Primary sinks in MI were distributed widely at 200-1200 μm. The relationship between the facial nerve-evoked FP map of MI and the output map of MI as revealed by intracortical microstimulation (ICMS) was examined in the same rats. Facial nerve-evoked potentials were obtained not only from the ICMS-defined vibrissal region of MI but also from adjacent regions including the forelimb, jaw and tongue regions. Furthermore, medial parts of the vibrissal region showed no or small responses to facial nerve stimulation.

Previous studies using electrical stimulation have demonstrated the role of the primary oral motor cortex in regulating oral behaviors. The present experiment examined changes in oral movements after direct pharmacological stimulation of the primary oral motor cortex in the awake restrained rat. Male Sprague-Dawley rats were implanted with bilateral injection cannulae (22 gauge) aimed at the primary oral motor cortex (A 3.5, L 13.5, V -1.5). Following one week recovery, animals were tested in observation chambers for motor activity. Infusion of N-methyl-D-aspartate (NMDA) and the GABA_A receptor antagonist, picrotoxin, produced a concentration dependent increase in oralofacial activity. Motor activity consisted primarily of non-directed chewing movements and tremor of the masseter muscles. In addition, both teeth grinding and directed biting were also observed. The effect was selective to oral behavior in that other motor activity did not show a significant increase. These findings demonstrate that a discrete orofacial motor syndrome can be directly elicited by pharmacological probes into the oral motor cortex. The relationship of these movements to subcortical oral motor pathways will be discussed. Supported by NIH DE09678.

EFFECTS OF REVERSIBLE COLD BLOCK OF LATERAL PERCERICAL CORTEX ON SWALLOWING IN AWAKE MONKEY. B.I. Sessle, N. Naito and R.E. Martin*. Fac. of Dentistry, Univ. of Toronto, Toronto, M5G 1G6 and Fac. of Appl. Health Sciences, Univ. of Western Ontario, London, N6G 1H1, Canada.

We have recently shown that swallowing can be evoked in the awake monkey by intracortical microstimulation (ICMS) of a region of the lateral pericentral cortex (Martin et al., Neurosci. Abstr. 15: 777, 1991). This study was initiated to determine the effects on swallowing of reversible cold-block induced inactivation of this ICMS-defined region. Two crucial areas were chronically implanted in the monkey (M. fascicularis). A warm or cold alcohol-water solution was pumped through thermoregulated bilateral catheterized sites overlying the lateral pericentral cortex while the monkey chewed standardized amounts of fruit during pre- cool (thermodome temperature 37°C), cool (0°C), and rewarm (37°C) conditions. Electrophysiological activity was recorded from motor (MA), premotor (PM), anterior digastric (AD), genioglossus (GG), posterior digastric (PD), and thyrohyoid (TH) muscles, and video recordings made of orofacial movements. The incidence of swallowing following chewing was significantly decreased during cold block (14.8%, t-test, p < 0.0001) compared to pre-cool (88.5%) and rewarm (100%) conditions. Swallow-mediated EMG activity following swallowing, twitching and licking was also affected, e.g. EMG bursts following evoked tongue movements were significantly reduced during cold block (mean ± SD: 0.75 ± 0.35s and 4.75 ± 1.11 A/D units, respectively) compared to pre-cool (0.40 ± 0.04s and 7.62 ± 0.68 A/D units) and rewarm (0.44 ± 0.05s and 7.06 ± 0.91 A/D units) conditions, and the interval between MA and TH swallowing-related EMG onset following swallowing significantly increased (0.18±0.06 A/D, ANOVA, p < 0.05) compared to pre-cool (0.11±0.03s) and rewarm (0.19±0.02s) conditions. These data provide further evidence that the lateral pericentral cortex plays a critical role in the initiation and regulation of swallowing in the primate. Supported by Canadian MRC grant MT-4918.

PROCESSING OF IPSILATERAL SOMATOSENSORY AFFERENTS IN PRIMARY MOTOR CORTEX OF THE MACAQUE. M.G. Lee* and L.C. Arcezo. Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Direct excitatory afferents from the contralateral hand to primary motor cortex have been demonstrated in single unit studies in primates. The contribution of activity evoked in the contralateral median nerve stimulation was explored to investigate potential excitatory or inhibitory interactions between the bilateral afferents in this area. Local populations of neurons in the hand representation area of 4 in two awake adult macaque monkeys were sampled with a low impedance (300-500kOhm), multi-electrode (150-200; 80-120; 100-150) spacing. Some evoked potentials (SEP's) for tibial nerve multiunit activity (MUA) were measured simultaneously from up to fifteen sites within and across laminae, and one-dimensional current source density patterns (CSD) were calculated. Stimulation of the contralateral hand produced an ipsilateral SEP characterized by an inverting potential, P17/N17, which consists of about 8 ms. This component is associated with a coincident excitatory burst of MUA which returns to baseline at approximately 50ms and is followed by a subtle reduction in MUA. Stimulation of the ipsilateral hand, however, elicits a markedly different response, characterized by a sustained increase in MUA at about 120ms. This is followed by a prolonged and striking 20-30% reduction below the spontaneous firing level in MUA from about 50 to 110ms. The laminar distribution of MUA and associated excitatory and inhibitory flow in the ipsilateral response is consistent with a pattern of excitatory input followed by the active hyperpolarization of apical dendrites of infragranular pyramidal cells. While the timing of activity suggests that the excitatory inputs are direct rather than collateral, the origins of the inhibitory activity have yet to be elucidated. These data support the hypothesis that primary motor cortex contains an inhibitory component of somatosensory inputs and suggest that the ipsilateral afferents may serve to sharpen the boundary between afferents from contralateral and ipsilateral hands. Supported by NIH NS 09723.

EFFECTS ON MASTICATION OF REVERSIBLE COLD BLOCK OF LATERAL PERCERICAL CORTEX OF AWAKE MONKEY. N. Naito, B.I. Sessle, B. Raaduf and C.-S. Huang.* Faculty of Dentistry, University of Toronto, Toronto, M5G 1G6, Canada.

Our laboratory has shown that intracortical microstimulation (ICMS) of a region of the lateral pericentral cortex can evoke masticatory-like movements in awake monkeys (Huang et al., J. Neurophysiol. 61; 635, 1989). The aim of this study was to determine if the effect could be reversed by reevoking cold-block induced inactivation of this ICMS-defined region. Two crucial areas were chronically implanted in the monkey (M. fascicularis). A warm or cold alcohol-water solution was pumped through thermoregulated bilateral catheterized sites overlying the lateral pericentral cortex while the monkey chewed standardized amounts of fruit during pre-cool (thermodome temperature 37°C), cool (0°C), and rewarm (37°C) conditions. Electrophysiological (EMG) activity was recorded from masseter (MA), anterior digastic (AD) and other orofacial muscles, and video recordings made of orofacial movements. The chewing cycle and preparatory phase of rhythmic chewing were significantly prolonged during cold block (mean ± SD: 0.56 ± 0.02s and 2.29 ± 0.96s, respectively, ANOVA, p < 0.05) compared to pre-cool (0.31 ± 0.06s and 0.75 ± 0.22s) and rewarm (0.32 ± 0.04s and 0.70 ± 0.15s) conditions. EMG burst durations were also significantly prolonged during cold block (MA: 0.35 ± 0.12s, AD: 0.33 ± 0.11s, ANOVA, p < 0.05) compared to pre-cool (MA: 0.23 ± 0.04s, AD: 0.19 ± 0.05s) and rewarm (MA: 0.25 ± 0.03s, AD: 0.20 ± 0.05s) conditions, and EMG amplitudes were also significantly reduced (MA: 0.82 ± 0.21; AD: 1.74 ± 0.48, A/D units, ANOVA, p < 0.05) compared to pre-cool (MA: 1.06 ± 0.24s, AD: 2.19 ± 0.43, A/D unit) and rewarm (MA: 0.98 ± 0.23, AD: 2.20 ± 0.63, A/D units). These data provide further evidence that the lateral pericentral cortex plays a critical role in the initiation and regulation of masticatory movements. Supported by Canadian MRC grant MT-4918.

MODULATION OF OPTICAL INTRINSIC SIGNALS BY INTRODUCTION OF A COMPETING STIMULUS. A.J. Blood and A.W. Tong. Laboratory of Neuro Imaging, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Optical intrinsic signal imaging was used to detect reflexive changes in two regions of rat somatosensory cortex. Possible interactions of activity between barrel cortex and the forelimb region of the primary somatosensory cortex were investigated. This was accomplished by evaluating intrinsic signal response magnitude and spatial extent in response to simultaneous peripheral stimulation of simple and crossed whiskers and forelimb digits. Whisker C1 was deflected at a frequency of 10 Hz for 1 second while vibratory stimuli of varying intensities were applied to forelimb digits. Intrinsic signal responses to simultaneous whisker and forelimb stimulation were compared to whisker stimulation alone. Addition of the forelimb stimulus modulated intrinsic signal activity by increasing or decreasing the magnitude and/or spatial extent of responses over barrel cortex. The degree of modulation was related to the intensity of the competing stimulus. These data suggest that cortical responses at the level of primary sensory processing may be significantly modified by activity in adjacent regions.


The response characteristics of vestibularly driven units in the parieto-insular vestibular cortex (PIVC) have been tested in four alert squirrel monkeys. Sinusoidal vestibular and optokinetic stimulation was applied in 8 different planes orthogonal to the yaw plane. Various forms of somatosensory stimulation were applied, such as brushing the skin, pressing different muscles, moving different joints. The neck-muscle input was tested by moving the body sinusoidally while the head was fixed in space. In the PIVC region of the squirrel monkey about 60 percent of the units were driven by vestibular stimuli. Frequently vestibular neurons also responded to neck-muscle stimulation, input from other muscles or tendon or joint receptor stimulation. One third of the PIVC-units responded to optokinetic or small-field visual movement stimulation. Polar histograms of spatial tuning of the vestibular driven units in the PIVC showed, as a rule, a sector of maximal response around a preclinical plane. Response planes did not correspond in most cases to the planes of the semicircular canals. Our results give evidence that the network of the PIVC cells can be interpreted as a cortical integrator of vestibular input from all semicircular canals. The PIVC processes information from the vestibular, somatosensory and optokinetic systems, which is useful in calculating the movement of the head in space and in relation to the other parts of the body.
MOTOR CORTEX: FUNCTIONAL ORGANIZATION AND PLASTICITY I

746.1

MOVEMENT REPRESENTATION IN PRECENTRAL MOTOR AND PREMOTOR CORTEX OF OLD WORLD MONKEYS. J.M. Preuss, I. Stepnowska and J.H. Kas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The lateral premotor cortex of Old World monkeys has not previously been examined in detail. To ascertain its microphotostimulation. We used this technique to map the distribution of motor responses across the precentral gyri in 8 Old World monkeys (7 Macaca spp., 1 Erythrocebus patas). Animals were anesthetized with ketamine and xylazine and current delivered with tungsten microelectrodes.

We were able to elicit responses reliably from both the dorsal and ventral premotor areas (PMd, PMV), identified architectonically, as well as from caudal and rostral divisions of the primary motor area (M1c, M1r). These divisions were distinguished from PMd or PMV, even in PMd, by consistent responses to either PMd or PMV, but even in PMd, many points were responsive with currents of 40-100 µA. We elicited hindlimb, axial, forelimb, and facial movements from both PMd and M1, but only forelimb and facial movements after stimulation of PMV. Thus, we found no evidence of hindlimb representation in PMV. The hindlimbs are bordered medially by a zone of facial and upper axial responsiveness located in lateral-most PMd. Stimulation yielded both proximal and distal forelimb movements from PMd, but predominantly distal movements from PMV. These results are largely consistent with our previous microstimulation studies in owl monkeys, indicating that stimulation of premotor cortex elicits response. Moreover, our results do not support the widely held view that there is a caudal-to-rostral gradient of distal-to-proximal axial representation in precentral cortex.

(Supported by NS 16446 and NSF 890-35).

746.2

ROLE OF SOMESTHETIC INFORMATION IN THE PLASTICITY OF THE CEREBELLO-CORTICAL PATHWAY. E.M. Mostafavi and L. Kaas. Dept. of Neurological Surgery, University of California, Irvine, CA 92617.

Long-lasting effects of somesthetic messages on cerebellum-thalamo-cortical (CCT) transmission and on the motor responses induced by interpositional nucleus stimulation, were studied under associative conditions. Two co-conditioning procedures, in association with behavioral and electrophysiological approaches, were carried out on awake chronically prepared cats which had previously undergone a red nucleus neurotoxic lesion. In the 1st procedure, a sub-threshold activation of the CTC circuits controlling forearm flexions movements (conditional stimulus CS), was associated with a cutaneous stimulation (unconditional stimulus UCS) of the domain of the dorsal forearm. Both CS and UCS originally produce elbow flexions. In the 2nd procedure, the same CS was paired with another UCS, applied to a more proximal cutaneous receptive field and producing a backwards forearm withdrawal movement, different from that induced by the CS.

Under the 1st procedure, a persistent enhancement of the amplitude of the forelimb flexions induced the CS was observed along with an increase of the cerebello-cortical excitatory responses in the elbow area of the motor cortex. In contrast, the 2nd procedure resulted in a decrease and the disappearance of the forelimb flexions in favor of the appearance of forearm extensions, the amplitude of which increased concomitantly with a depression of the cerebello-cortical excitatory wave. A secondary inhibitory wave also developed in the cortical elbow area. These changes were long lasting and lead to the following conclusions: The CTC circuits are liable to undergo functional modifications in adult cats. The motor and CCT changes may be of two types depending on the somesthetic messages paired with the CS. The time courses of the changes affecting the CTC transmission and the motor responses are closely related.

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747.1


In the present study γ-aminobutyric acid (GABA) immunoreactivity was evaluated quantitatively in the hindlimb representation of the rat somatosensory cortex after 14 days of tail suspension (TS). The number of GABA-immunoreactive cells was reduced in cortical layers IV, Va, Vb and VI (p<0.05, p<0.0002, p<0.02, and p<0.03 respectively) of rats subjected to TS. In addition, the number of GABA-containing terminals, particularly those terminals surrounding the soma and apical dendrites of pyramidal cells in layer Vb, also were reduced throughout the same cortical layers. Since there was no reduction in the total neuronal density of the hindlimb representation as compared with the control animals, we concluded that the reduction was not due to cell death. Findings are discussed in the context of previous morphological and behavioral studies of the neuropeumatoc system of TS animals and we propose that alterations in the reflex organization of hindlimb muscle groups that are triggered by TS elicited disturbances in the afferent signaling and feedback from intramuscular receptors to the cerebral cortex. We suggest that local circuit GABAergic neurons modulate cortical output in response to this altered afferent feedback.

Supported by NASA Cooperative Agreement NCC 2-449.

747.3


The properties of cerebellar output pathways can be modified by sensorimotor experience during the lifetime of an animal. It would appear that this plasticity depends on the convergence of motor-related neural activity with sensory information concerning the environment. The present study was aimed at elucidating the importance of the temporal relationships between such sensory and motor events in the development of these experience related changes. In chronic cats, the cerebello-thalamo-cortical and cerebello-cerebellar motor circuits controlling the forelimbs were subjected to an associative conditioning procedure in which the interpositus nucleus (origin of both pathways) was subjected to sub-threshold conditioned stimulation in association with a peripheral stimulus (TS) which induced a reflex flexion movement of the elbow joint. The movements produced together with the responses in central structures were analysed during the course of the conditioning procedure. Three sets of experiments were tested. When the CS was presented 100 ms after the UCS (CS-UCS 100) there was no effect on either the movement or the central responses. When the CS was applied either at the same time as the UCS (CS-UCS 0) or 100 ms before (CS-UCS 100), there was an increase in both the amplitude and the probability of the motor responses. In the CS-UCS situation, the motor changes were only transient, and depended on changes in excitability that lasted only for the entire motor cortex. In contrast, in the CS-UCS 100 situation, the changes were topical and long-lasting and were associated with a reinforcement of thalamo-cortical and cerebello-rubral synapses. Such changes were specific to circuits involving the elbow joint and were strongly correlated with the changes in the movement. They were only seen when the CS predicted the arrival of the UCS.

747.4


Magnetocorticographic (MEG) somatosensory and motor fields of the left hand (mostly middle finger) were recorded in five healthy subjects before, during and after nerve conduction block of their forearms using a pneumatic cuff. In addition, in two subjects the MEG-recording was repeated after total lidocaine block of the left middle finger. During the first 15 minutes of ischemia a 50% decrease of amplitudes of the sensory fields parallelled the progressive sensory loss. Surprisingly, a reduction in the motor field occurred as well. Source locations of the primary motor field components shifted within an area of approximately 1 cm². This occurred in all subjects and had a characteristic direction for each individual. Further attenuation of field components was seen in the following 15 minutes of ischemic nerve block. Frequency spectrum analysis showed a suppression of motor mu rhythm as well as a reduction in the 4 Hz band during this period. The fields regained their baseline coordinates and amplitudes 15 minutes after cuff removal. Lidocaine had effects on amplitudes, frequencies and dipole locations selectively on the deafferentated middle finger similar to those of ischemia-induced anesthesia, while the non-anesthetized neighboring finger showed normal results. These findings imply that the ischemic effects are not due to cuff-related peripheral anoxia. The decrease of amplitudes in the motor field components during ischemia may be explained by reduced sensory afferent background activity. In summary, temporary peripheral deafferentation, either due to ischemic nerve block or to lidocaine-related digital block, leads to a rapid change in the frequency structure of sensory and motor field components.
MOTOR NEUROMAGNETIC ACTIVITY DURING TRANSIENT DEAFFERENTATION. R. Kristeva-Feige, S. Ross, V. Pizzella, A. Sabato, W.-F. Liu, W. Doehaart, J. Edrich and P.-M. Poslusn, Inst. Biomed. Eng., Univ. Ulm, 89069 Ulm, Germany. (SPON: EUROPEAN BRAIN AND BEHAVIOUR). The present study was aimed at investigating the effect of blocking cutaneous input from the moving part of the body on movement-evoked field one (MEF). Neuramagnetic fields from the left cerebral hemisphere of three healthy, right-handed subjects were investigated preceding and accompanying voluntary right hand movements performed under two different experimental conditions: before (stage A) and during (stage B) transcranial deafferentation. The last was achieved by blocking of median and radial nerves at wrist. In this way, during stage B cutaneous and part of the proprioceptive inputs relative to a wide hand area including the entire index finger were suppressed while voluntary movements were not impaired because muscles participating in the task were not influenced by the anesthesia. The magnetic signals of the brain sources corresponding to the main components of the movement-related fields (motor field, MF and movement-evoked field 1, MEF) were mapped and localized by means of a moving dipole model. In the three subjects investigated, the MF and MEF dipole sources in stage B were stronger (30% on average) than before anesthesia. No significant changes in component latencies and spatial co-ordinates of the estimated dipoles sources between stages A and B were observed.

The results are discussed in terms of three hypotheses not mutually excluding each other: “reference copy” hypothesis, spatial attention hypothesis and plasticity hypothesis.

747.7 NMDA RECEPTOR ANTAGONISTS REVERSIBLY BLOCK PLASTICITY OF ADULT MONKEY MOTOR REPRESENTATIONS. G.W. Honthorst1, E.J. Plautz,2, and R.J. Nudo,1 Department of Neurobiology and Anatomy, University of California, Irvine, CA 92717. NMDA receptors have been strongly implicated in forms of synaptic plasticity such as LTP, but any role they may play in the mechanisms contributing to activity-dependent changes that underlie maps is unknown. To investigate the role of NMDA receptors in motor cortex plasticity, we utilized a previously described paradigm (Sanes et al., PNAS 85:1998) in which tracation of the facial nerve induces a rapid expansion of the cortical forelimb representation into the former whisker representation. The motor cortices of adult rats were first mapped using low-threshold stimulation techniques to evoke movements, then exposed to antagonists (D-APV or MK-801) via slow release from the polymer Elvax (DuPont) implanted subcutaneously. After a 2-7 day exposure period, the contralateral facial nerve was cut, and 2-4 hours later the Elvax was removed and the cortex was immediately re-mapped. Forelimb movements were evoked reliably from all previously mapped forelimb representations at comparable thresholds, but no movements could be evoked from the previously mapped whisker region even at high stimulation currents (60-80 μA). After a 4 hour saline-drain to wash out the drug, the motor cortex was again re-mapped, at which time low-threshold forelimb movements were evoked from all previous forelimb positions as well as new spots corresponding to regions of the former whisker representation. The current thresholds were not statistically different from those obtained in the first mapping. Motor cortices exposed to control solutions (saline or the inactive stereoisomer L-APV) exhibited a forelimb border shift at the first mapping immediately after facial nerve cut. These data suggest that NMDA receptors may be a critical component of the synaptic connections which underlie rapid changes in cortical representational maps, and their functional activation may emerge under conditions in which plasticity is induced. Supported by grants from the Aaron Diamond Foundation, the Sinsheimer Foundation and the NS21377.

747.8 DIFFERENTIAL EFFECTS OF SKILL ACQUISITION AND MOTOR USE ON THE REORGANIZATION OF MOTOR REPRESENTATION IN A FLIXMIRE BOARD EXPERIMENT IN ADULT SQUIRREL MONKEYS. E.J. Plautz1, G.W. Milliken2, and R.J. Nudo,2 Dept of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225. Previous research has shown that training on a primary motor cortex (area 4) are alterable by behavioral experience (e.g., Milliken, et al., 1992; Nudo, et al., in press). In the present study, we examined effects of motor skill acquisition from effects of motor use. Intracortical microstimulation techniques were used to derive detailed maps of movement representations of the hand, wrist, and arm in area 4 before and after two types of training. Monkeys were trained to retrieve food pellets from either a small well (9.5 mm diameter) or a large well (19 mm diameter board). Mean of the standard deviation was assessed by: a) average number of digit flexions per pellet retrieval and b) frame-by-frame video analysis of movements and movement combinations made during pellet retrieval. Training continued until a criterion level of performance had been performed. Monkeys trained on the small well displayed poor performance initially but rapidly improved during the training period. Improvement in behavioral performance was paralleled by a shift in movement patterns. By contrast, monkeys trained on the large well exhibited nearly flawless retrieval performance throughout the training period and little variation in movement patterns over time. A third group of control subjects were not exposed to the training task. Following small well training, post-training maps revealed small expansions in the representation of digit movements and digit/wrist combinations. These expansions closely paralleled the movements used during successful task performance. In contrast, no systematic changes were found in digit or wrist representations following long well training, despite an equivalent number of total digit flexions. Similarly, control subjects showed little variation in movement representations between mapping sessions. These findings suggest that the reorganization of motor representations in area 4 reflects the acquisition of new motor skills, and does not simply reflect changes in motor use.

This work was supported by NIH NS03966 (GWM) and NS2794 (RJN).

747.9 RECOVERY OF FINGER MOVEMENT REPRESENTATION AFTER DISTAL FORELIMB RESTRICTION IN ADULT SQUIRREL MONKEYS. G.W. Milliken1, E.J. Plautz, and R.J. Nudo,1 Departments of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225. Using intracortical microstimulation techniques, detailed maps of movement representations in primary motor cortex (area 4) were derived before, during, and after resolution of the functional forelimb. Complete reorganization of the functional topography of motor cortex involved decreases in finger and increases in wrist representation. Three subjects demonstrated this pattern. In one subject, following a 4 week period of movements that involved only the more proximal fingers, movement representations in the primary motor cortex were no longer visible; indeed, extension of the movements to involve movements of the fingers produced no obvious response. These data suggest that the use of means to destroy the movements of the fingers may block recovery of movement representations. Recovery of movement representations in the primary motor cortex was related to behavioral use of the PRP hand, and that recovery can occur with forced rehabilitation.

Supported by NIH NS03966 (GWM) and NS2794 (RJN).

747.10 LIMITED MOTOR CORTEX REORIENTATION IN A LONG-TERM MONKEY AMPUTEE. M.A. Schieber1, V. H. Andersen1, and R.K. Dreyer1 Department of Neurology and Neurobiology, University of Rochester, Rochester, NY, 14642 and Washington University, St. Louis, MO, 63110. After forelimb amputation in neonatal rats, the primary motor cortex (M1) reorganizes such that stimulation in the previous forelimb region can evoke movements of the face (Donoghue & Sanes, 1988). But in adult rat or human amputees, stimulation of the M1 region at the extreme edge of the arm representation did not evoke movements of the forearm, which may involve contractions of remaining shoulder stump muscles (Sanes et al., 1990; Cohen et al., 1991). We studied M1 in a 15 year old monkey that for unrelated reasons prior to age 2 had undergone amputation of the right arm at the shoulder joint. We used intracortical microstimulation (ICMS) to explore the region of M1 normally devoted to the arm and hand bilaterally, and to identify its borders with the face region laterally and the leg region medially. In the right M1, conventional ICMS evoked movements of the left face, arm and leg at current thresholds at or below 40μA; the locations of the face/arm and arm/leg borders were normal. In the left M1 cortex only a very small region of the face and arm border area evoked movements of the right face, right arm and leg at high current thresholds at or below 40μA; the location of the face/arm and arm/leg borders were normal. In the left M1 cortex only a very small region of the face and arm border area evoked movements of the right face, right arm and leg at high current thresholds at or below 40μA; the location of the face/arm and arm/leg borders were normal. In the left M1 cortex only a very small region of the face and arm border area evoked movements of the right face, right arm and leg at high current thresholds at or below 40μA; the location of the face/arm and arm/leg borders were normal.

Modified ICMS using currents up to 80μA and trains up to 200ms also failed to evoke movements of the right face, neck, abdomen or leg, or of the left arm. The M1 arm region contralateral to the amputated arm showed no reorganization to represent the face or leg. Instead, shoulder stump movements were evoked throughout the arm region, which may have resulted from unmasking of the normal widespread shoulder representation. This age at amputation, and/or time since amputation, may affect M1's reorganization. Support: NINDS R01-NS27686 to MHS, NSF 921-0237-09 to RFK, McDonnell.

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747.11 LEARNING AND RETENTION OF A NECTORALLY-DEPENDENT SENSORMOTOR SKILL IN THE RAT. Michael Coogan, John Lason, & Gary Lynch. Dept. of Anatomy, Univ. of CA, Irvine, CA 92717.

Traditional models of cortical control of fine, distal limb movements have typically been limited to primate species. However, rats can be trained to use their forepaws to grasp and retrieve small pellets, and this behavior is similar to the manipulative behaviors of primates. Neuromechanical and behavioral studies support the notion that rats can serve as a model of primate motor skill learning and performance, and that the normal and pathological reach behaviors of rats are likely to be directly applicable to the understanding of human cortical pathophysiology. The present experiments describe a paradigm for examining cortical and subcortical motor control of the hindlimb in rats.

Adult, male Sprague-Dawley rats were trained to reach for food rewards in an apparatus originally designed by Montoya et al. (1991). This apparatus provides for varying degrees of difficulty of the task and is used to examine the electrophysiological and morphological properties of neurons in long-term (>4 weeks) co-cultures. Furthermore, as local interneurons are found to profoundly influence cortical and striatal dynamics, a special effort was undertaken to reconstruct those interneurons.

Cortical and striatal slices from rats P0 - P1 were cultured for four to eight weeks using a modified roller-boat technique. Neurons were intracellularly recorded from, electrophysiologically identified and labeled with neurobiotin.

In the cortex, pyramidal neurons, bipolar and multipolar interneurons show similar dendritic and axonal morphologies as in vivo. In the striatum, the giant aspiny interneuron class, two GABAergic interneuron classes and medium-spiny projection neurons can be clearly distinguished from each other and are similar to in vivo. These results indicate a high degree of similarity in the electrophysiology and morphology between neurons in long-term slice cultures and in vivo.

Supported by 'Deutsche Forschungsgemeinschaft' and USPHS grant NS 20702.

748.1 LONGTERM CORTICAL AND STRIATAL SLICE CO-CULTURES: ANATOMY AND ELECTROPHYSIOLOGY OF IDENTIFIED NEURAL TYPES. B. Tang, D. Perez, Y. Kuga* and S.T. Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

To study cortico-striatal dynamics in vitro system has been developed using organotypic cortex-striatum co-cultures. Previous work showed that the spontaneous activity developing in this system proves to be highly similar to the ones found in vivo. Here we further examine the electrophysiological and morphological properties of neurons in longterm (> 4 weeks) co-cultures. Furthermore, as local interneurons are found to profoundly influence cortical and striatal dynamics, a special effort was undertaken to reconstruct those interneurons.

Cortical and striatal slices from rats P0 - P1 were cultured for four to eight weeks using a modified roller-boat technique. Neurons were intracellularly recorded from, electrophysiologically identified and labeled with neurobiotin.

In the cortex, pyramidal neurons, bipolar and multipolar interneurons show similar dendritic and axonal morphologies as in vivo. In the striatum, the giant aspiny interneuron class, two GABAergic interneuron classes and medium-spiny projection neurons can be clearly distinguished from each other and are similar to in vivo. These results indicate a high degree of similarity in the electrophysiology and morphology between neurons in long-term slice cultures and in vivo.

Supported by 'Deutsche Forschungsgemeinschaft' and USPHS grant NS 20702.

748.2 CORTEX AND STRIATUM SLICE CO-CULTURES: POPULATION ACTIVITY UNDERLIES HIGH FREQUENCY SUBTHRESHOLD OUTFLOW NEURONAL RESPONSES. S. Kuga and S.T. Kitai*. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

It has been hypothesized that a brief synchronization of spike discharge among a group of neurons is one of the basis for neuronal coding and among cortical neurons has been observed to be ~40 Hz. However, the relation between neuronal information processing and the underlying mechanisms of synchronized spike discharge, we have established longterm (4-8 weeks) organotypic cortex-striatum co-cultures from rats at age P0 - P1. Simultaneous intracellular recordings were obtained from neurons in the cortex, the striatum or both. Neurons were identified based on their electrophysiological characteristics and their morphological features were revealed by intracellular labeling with neurobiotin.

Findings indicate: (1) In the cortex during periods of spontaneous activity, brief (0.5 - 1.5 s) ~40 Hz subthreshold oscillations were observed to which pyramidal neuron spike discharge is temporally linked. (2) In the striatum, medium-spiny neuron spike discharge is temporally linked to > 20 Hz subthreshold oscillations. (3) In both structures, spike discharge of GABAergic interneurons was closely associated with subthreshold oscillation frequency. (4) These results indicate that: (1) The ~40 Hz subthreshold oscillation in the cortex is an emergent property of the cortical network itself. (2) Burst activities (~20 Hz) of striatal medium-spiny projection neurons may be governed by the striatal network, which is driven by cortical inputs. (3) Subthreshold oscillation frequencies in each structure result from population activity in which local GABAergic interneurons strongly participate.

Supported by 'Deutsche Forschungsgemeinschaft' and USPHS grant NS 20702.

748.3 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF IMMUNOCYTOCHEMICALLY IDENTIFIED NEURONS IN THE RAT SUBSTANTIA NIGRA PARS RETICULATA IN VITRO. C.D. Richards, T. Shirayama* and S.T. Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Previous studies on the substantia nigra pars reticulata (SNr) have identified two groups of neurons based upon their electrophysiological characteristics. However, the transmitter phenotype of each of these two neuronal classes is unknown. The aim of this study was to record from neurons in the SNr and characterize them electrophysiologically, and subsequently to identify these neurons using immunocytochemical double labeling techniques.

Intracellular recordings performed in slices of midbrain containing the SNr, prepared using standard techniques. Electrodes contained neurobiotin, which was injected into neurons subsequent to electrophysiological characterization. Two electrophysiological classes of neurons were identified. One had a slow rate of action potential discharge, long duration action potentials, and prominent anomalous inward (Ih) and transient outward (Ito) rectification. The other had a faster rate of action potential discharge, shorter duration action potentials, weak Ih, and no apparent Ito.

Both types of neurons were subsequently processed immunocytochemically to reveal the injected biocytin. The former type of neuron, which had electrophysiological characteristics similar to substantia nigra pars compacta neurons, displayed double labeling for biocytin and tyrosine hydroxylase, indicating that they are dopaminergic. The latter, faster firing type of neuron is currently being processed in order to determine if these neurons lack the presence or absence of glial fibrillary acidic protein (GFAP), the marker enzyme for GABAergic neurons.

This study indicates that the SNr contains two classes of neurons which can be distinguished using intracellular recording and electrophysiological criteria.

Supported by USPHS grants NS 20702 and NS 25473.

748.4 A FURTHER CHARACTERIZATION OF RAT TEGMENTAL PEDUNCULOPONTINE (PPN) CHOLINEnergIC NEURONS. K. Takahashi, T. Shirayama and S.T. Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

We have demonstrated that PPN Type II neurons are cholinergic and characterized by a transient outward current in in vivo slice preparations (Kang and Kitai, Brain Res. 1990). In this study, Type II neurons (n=69) were classified into 2 subgroups according to their electrical membrane characteristics. One group (short-duration neuron; SDNs, n=21) was characterized by short duration spikes (0.7-1.3 ms), a high spontaneous firing rate (n=14 Hz) and high input resistance (n=15.9 MΩ).

Depolarizing current injection increased the firing rate of these neurons with no alterations in duration or fast-AHP of spikes. The other group (long-duration neurons; LDNs, n=48) was characterized by long duration spikes (1.6-2.8 ms), a low firing rate (n=7.8 Hz) and low input resistance (n=140 MΩ). They displayed accommodation of firing frequency with changes in spike duration and fast-AHP. In the presence of TTX (<10 μM), SDNs displayed Ca-dependent high threshold oscillations (HTO) with high frequency (n=15.3 Hz) and low voltage (n=2.8 mV). LDNs had low frequency (n=7.5 Hz) and large amplitude (n=5.2 mV) HTO. An application of TEA (5 mM) generated spontaneous high threshold Ca-spikes in LDNs but not in SDNs. Biocytin injection combined with chAT immunohistochemistry revealed 40% of each subtype of neurons were cholinergic.

Morphologically, SDNs had small cell bodies (<20 μm) with 3-4 thin primary dendrites and LDNs large cell bodies (>25 μm) with 3-6 thick primary dendrites. Both groups of cells were intermingled within PPN. These results suggest that PPN cholinergic neurons are composed of heterogeneous subgroups of neurons with different membrane characteristics and morphology. Supported by USPHS grants NS20702 and NS25473.
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forebrain
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This
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was
supported
by
NIMH
grant
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SOMA-AND-DENDRITIC
DOPAMINERGIC
(RECEPTOR-MODULATED
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NUCLEUS-ACCUMBENS
NEURONS
IN
VITRO.
Gundersen,
Y.
N.,
E.
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C.,
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Kleinschmidt,
J.
S.,
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of
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DOPAMINE
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V
VICINAL
CORTEX-
STRATIUM
NEURONS
EVIDENCED
BY
SYMPATHETIC
ACTIVATION
OF
THEIR
APICAL
DENDRITES
IN
VITRO.
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Yang,
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Seamans,
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Activation
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currents
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electrotonic
depolarization
of
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dendritic
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produces
a
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of
calcium
entry
through
dendritic
DADLE
channels.

478.9
ENCEPHALIN
INHIBITS
IMMEDIATE-EARLY
GENE
EXPRESSION
INDUCED
BY
BLOCKADE
OF
d2
DOPAMINE
RECEPTORS
IN
STRIATUM.
H.
Steiner,
R.
Maitori,
and
C.
R.
Gerfen.
Laboratory
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MD
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in
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We
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immediate-early
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zif268
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fos.
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SYSTEMIC
BUT
NOT
INTRASTRIAL
INJECTION
OF
THE
MUSCARINIC
AGONIST,
OXITREMORINE,
INDUCES
CORTEX
AND
STRIATUM
IMMEDIATE-EARLY
GENE
EXPRESSION.
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Nakamura,
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Kaneko,
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Gerfen,
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Neurobiology,
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Virginia,
Charlottesville,
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Muscarinic
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Dopamine
receptors
in
the
striatum.
This
work
was
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by
NIMH
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"Society
for
Neuroscience,
Volume
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1995"
748.11 STRIATAL EXPRESSION OF ZIP26 mRNA TO ACUTE SHT-2A AND SHT-2C RECEPTOR STIMULATION AFTER UNILATERAL LESIONING OF THE NIGROSTRIATAL PATHWAY. R.F. Faleski and G. R. Oemus Laboratory of Systems Neuroscience, NIMH, Bethesda, MD 20892.

The striatum receives a dopamine projection form the substantia nigra and the ventral tegmental area via the dorsal raphé. Whereas dopamine differentially modulates the function of connectionally distinct striatal neurons, the influence of serotonin on striatal neurons is less understood. In particular, the effect of serotonin, via the SHT-2A and SHT-2C receptors, on striatal neuron function was investigated in the presence and absence of endogenous dopamine input. Rats were given unilateral injection of SHT-2A or SHT-2C, increased expression of ZIP26 in the lesioned striatum, while producing smaller changes in the intact striatum. mCPP, which has higher affinity for the SHT-2C receptor, significantly reduced ZIP26 mRNA expression in the lesioned striatum, but had little effect on the intact side. These data indicate that serotonin differentially affects striatal neuron function via the SHT-2A and SHT-2C receptors. The SHT-2A receptor appears to increase striatal neuron function, whereas the SHT-2C receptor appears to decrease striatal neuron function. Furthermore, these data demonstrate that dopamine can reduce the response of striatal neurons to serotonin receptor stimulation.


We have previously reported (Szele et al., J. Neurosci., in press) that expression of GAP-43, an axonal protein required for both axonal growth and sprouting axons, is decreased in the striatum after unilateral lesions of the sensory-motor cortex induced by acute aspiration, but not after progressive lesions induced by thermocoagulation of pal blood vessels. We have examined GAP-43 immunostaining at the ultrastructural level in the striatum of rats 16 days after both types of lesions. Both lesions induced similar loss of all layers of the sensory-motor cortex. In control rats, GAP-43-immunoreactivity in the dorsolateral striatum was most often associated with axon terminals enriched in various vesicle types (GABAergic, glutamatergic, and GABAergic). Processes these processes contained different asymmetric contacts in dendritic spines, GAP-43 immunoreactivity was also present in pre- and terminal segments of axons, and to a lesser extent in axon terminals forming asymmetrical and symmetrical synapses and in dendritic spines. After aspiration lesions, extensive degeneration was present in the denervated dorso-lateral striatum; GAP-43 immunoreactivity was undetectable, and growth cone-like axon terminals were absent. In contrast, expression of GAP-43 and growth cone like axon terminals similar to those seen in controls were present in the dorsolateral striatum after thermocoagulation cortical lesions. Together with data showing increased labeling of crossed corticostriatal projections after thermocoagulation lesions (Napieralski et al., this meeting), the data suggest that compensatory axonal sprouting occurs in the dorsolateral striatum after thermocoagulatory but not aspiration cortical lesions. Sup. by NSF92230.

748.13 A ROLE FOR CONTRALATERAL CORTICOSTRIATAL NEURONS IN THE EFFECTS OF THERMOCOAGULARY LESIONS OF THE CORTEX ON STRIATAL GENE EXPRESSION. J.A. Napieralski, A.K. Butler, and M.F. Chevallier, Dept. of Pharmacology, University of Pennsylvania, Phila, PA 19104.

Previous studies in our laboratory have shown that cortical lesions induced by thermocoagulation of pal blood vessels, but not by acute aspiration, result in a decrease in expression of GAP-43 and GAP-43 expression in the ipsilateral denervated cortical lesion. We have examined whether contralateral corticostriatal projections from the spared homotypic corticostriatal corticostriatal projections contribute to these effects. Adult rats received a unilateral lesion of the cerebral cortex and, after thirty days, received intraperitoneal injection of the anterograde tracer, Ruby red, in the contralateral homotypic cortex. Rats were sacrificed 7 days later and labeled fibers were examined with fluorescence microscopy in the ipsilateral and contralateral corticostriatal projections. The results were consistent with labeling in the lesioned and unlesioned rats. Numerous labeled fibers were detected in the contralateral striatum of lesioned but not control animals, suggesting that contralateral cortical corticostriatal neurons may undergo axonal sprouting in the denervated striatum. To determine whether contralateral corticostriatal corticostriatal projections play a role in the expression of GAP-43 in the striatum induced by the cortical lesion, the effects of unilateral and bilateral thermocoagulation of the cortex were compared. In situ hybridization histochemistry revealed an increase in the expression of GAP-43 mRNA levels in the striatum of unilaterally but not bilaterally lesioned animals, supporting that sprouting or over activity of contralateral corticostriatal input is responsible for the increase seen after unilateral lesion. Supported by PHS grants NS-29100 and MH10794.

748.14 TOPOGRAPHICAL PATTERNS OF EFFECTS FROM THE 'BARREL' CORTEX TO RAT NEOSTRIATUM, A.K. Wright, D.M. Munro, L. Norrie, C.A. Ingham, G.W. Arbuthnott Staff, University of Edinburgh Centre for Neuroscience, Preclinical Veterinary Sciences, Summerhall, Edinburgh, EH9 1JH.

Individual whiskers on the face of rats are represented by an area of 1 mm cortex called a 'barrel'. Individual barrels, identified by cytochrome oxidase histochemistry, have been injected in pairs with different anterograde tracers and the projections of neurons within single barrels into the neostriatum, thalamus and brain stem examined.

We have used biotinylated dextran amine (BDA) and Phaseolus vulgaris Leucoagglutinin (PhaL), to fill anterograde projections in the same barrel. After 48 hour survival time have stained the two tracer different colors with 'Vector' chromagens DAB and SAG. The axons from the injected cells can be followed into the internal capsule from which they exit as large fibre tracts to supply small areas within the homunculus field of the neostriatum. Axons continue within the internal capsule fibres to other areas of brain including thalamus and brain stem.

There is a topographically arranged pattern of barrels in the dorsolateral portion of the striatum with very little overlap between fibres from individual barrels. Barrels of row A are closest to the caudal pole while those of row B are deepest in the striatum. A pattern of finer terminal is also visible which may have a different source within the barrel cortex and which seems to have a different topography.

Electron microscopic investigation shows both large and small sized complex synapses with dendritic spines and smaller fibres making simple contacts individual spine heads. In view of the likely loss of synapses of the asymmetric type when dopamine is lost from the striatum (Ingham et al. in press Br. Res. 93:17) it will be important to compare the fate of these synapses after 6-hydroxydopamine treatment.

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Cocaine and amphetamine are psychostimulant stimulants that exert potent acute and chronic effects on behavior. A candidate mechanism for mediating the long-term effects is the induction of cascades of regulation, including regulation of immediately early genes (IEGs) coding transcription factors and genes coding neuropeptidergic neuropeptidergic neuropeptides such as encephalins and dynorphin. Pharmacological experiments have implicated D1-class dopamine receptors in many of these effects, but action of other dopaminergic (e.g. dopamine D2-class and SHT) have also been suggested. We have examined whether, in such D1 receptor responsive may induce IEGs in the striatum. Whole-brain (wr) and mutant mice were given cocaine (40 mg/kg) or amphetamine (10 mg/kg) under single- or hindlimb conditions, and 2 or 3 days later the stained for c-fos and Jun B-like proteins in the striatum of mice in drug treated and control mice. Mutant and control mice were untreated or given haloperidol (2 or 5 mg/kg). The results were clear-cut. Cocaine and amphetamine strongly induced c-fos and Jun B-like proteins in the striatum of mice in drug treated mice as well as in wt mice. We conclude that the D1 receptor is essential for striatal induction of c-fos and Jun B-like proteins by cocaine and amphetamine. Deficits in IEG induction could contribute to the neurobehavioral deficits in the mutant mice and to their behavioral abnormalities as well. Supported by NIDA DA08307.

749.2 WITHDRAWAL FOLLOWING REPEATED AMPHETAMINE TREATMENT INCREASES DYE COUPLING BETWEEN NEURONS AND ACTIVATES OUTPUT PATHWAYS IN RAT LIMBIC CORTICAL/STRIATAL REGIONS S.P. Ona* and A. A. Grace, Depts Neuroence and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Although the mesocortic dopaminergic (DA) system has frequently been implicated in behavioral sensitization, its cellular or synaptic mechanisms are unclear. Using in vivo intracellular recordings and Lucifer-yellow dye injection, we examined the impaired or enhanced synaptic sensitization on dye coupling between dopaminergic post synaptic targets neurons in rat striatum and cortical regions. Following amphetamine treatment (2.4 mg/kg, i.p., 2 daily, 1.4 weeks) showing that withdrawal, significantly higher levels of dye coupling were observed between projection neurons in the infralimbic/parolfactory cortex (cAMP 5:6 vs basal 0:9) as well as within the ventral striatum, anterograde and amphetamine (mg/kg, 2x6). The increased coupling observed following aRPM was similar to that following serotonin depletion of striatal DA (2:16). Nevertheless, the density of tyrosine hydroxylase (TH) immunoreactive terminals in the striatum was not substantially altered in the rAmp-treated rats. Thus, these combined data suggest that withdrawal following aRPM produces a functional desensitization of the DA system without causing a actual loss of DA terminals. Furthermore, activity within the ventral cortico/striatal output pathways, as revealed by c-fos immunoreactive levels, showed an elevation above control levels in the rats following aRPM withdrawal. (Support by NARSAD Young Investigator Award and NS19608, MH44317 & 45166.)
749.3  
CANNABINOID AGONIST WIN 55212 MODULATES STRIATONIGRAL NEUROTRANSMISSION BY A PRESYNAPTIC MECHANISM. 


The brain cannabinoid receptor has been shown to be present in areas receiving striatally innervated, including the substantia nigra (SN), and to be located presynaptically on the GABAergic striatal output neurons. This study tested the hypothesis that cannabinoid agonist, applied systemically, could modulate striatognral transmission, but we did not affect the response of the postysynaptic agonist to locally-applied GABA. Male Sprague-Dawley rats were anesthetized with chloral hydrate. A glass micro-electrode was used to record the spontaneous activity of SN neurons, to lopomorphically apply GABA, and to pressure eject WIN 55212, a cannabinoid agonist, in vehicle. This electrode assembly was lowered into the SN and SN neurons were identified by the firing rate and shape of the action potential. Recording sites were marked for histological confirmation. WIN 55212 increased the spontaneous neuronal firing rate by 30-46%. The peak effect of WIN 55212 was reached 4.5-8.5 minutes after onset of pressure ejection. The percent inhibition of neuronal activity produced by GABA was not altered in the presence of WIN 55212, indicating that WIN 55212 had no effect on the interaction of GABA at the postynaptic membrane. Pressure ejection of vehicle, 45% 3-hydroxypropyl-β-cyclodexlein, produced a small, non-significant decrease in spontaneous neuronal discharge, and no change in response to GABA. These findings suggest that cannabinoid receptors on the terminals may modulate striatognral neurotransmission presynaptically. Supported by NIH grant DA 02194.

749.4  
PHYSICAL WITHDRAWAL IN RATS TOLERANT TO Δ2-THC PRECIPITATED BY A CANNABINOID RECEPTOR ANTAGONIST. 

Kang Zuo, Sanada L. Patrick, Russell M. Church, and J. Michael Walker. Schrier Research Laboratory, Departments of Psychology and Pharmacology, Brown University, Providence, RI 02912.

Whereas termination of the administration of opiates, barbiturates, and certain other psychoactive drugs leads to serious physical withdrawal symptoms, discontinuution of any heavy use of marijuana leads to relatively minor symptoms in animals and humans. This is perhaps due in part to the slow elimination of marijuana's main psychoactive ingredient, Δ9-tetrahydrocannabinol (Δ9-THC), as well as the continuous presence of cannabinoid receptor antagonists in the brain. To test the hypothesis that rapid termination of interactions between Δ9-THC and cannabinoid receptors would lead to withdrawal symptoms in tolerant animals we preincubated with the newly-developed competitive cannabinoid antagonist SR141716A. Tolerance to Δ9-tetrahydrocannabinol (Δ9-THC) was produced in rats by twice daily injections (15 mg/kg i.p.) for 6.5 days; a separate group of animals received an injection of the vehicle or SR141716A. This drug produced few effects in animals with previous injections of the vehicle only. By contrast, SR141716A induced a profound precipitated withdrawal syndrome in tolerant rats. The syndrome was characterized by constantly changing brief sequences of behavior resulting in a hyperactive appearance but lacking the stereotyped sequential and movement seen hyperactive during the hyperactive state produced by psychostimulant drugs. The withdrawal syndrome began about 10 minutes after injection of the antagonist and lasted for one to two hours. THC-resistant animals that were treated with vehicle remained quiet throughout the observation period. Administration of the antagonist i.e. to tolerant animals without withdrawal symptoms, indicates that the site of action of the antagonist for the most profound behavioral changes lies outside the periventricular core of the brain. These findings demonstrate that following long-term use, rapid discontinue of cannabinoid agonist/receptor interactions leads to profound withdrawal symptoms.

749.5  
ELECTROPHYSIOLOGICAL EFFECTS OF A CANNABINOID ON NEURAL ACTIVITY IN THE GLOBUS PALLIIDUS. 

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The high density of cannabinoid receptors in the globus pallidus and the motor effects of dopamine in the globus pallidus have suggested that the cannabinoid receptors in the globus pallidus may be important for the regulation of movement in the brain. Extracellular single neuron electrophysiology was used to explore the role of cannabinoids on neural activity in the globus pallidus. The synthetic cannabinoid WIN 55,212-2 (0.0625 - 0.5 mg/kg, i.v.) dose-dependently decreased the basal firing rate of neurons in the globus pallidus. This effect was not observed following administration of the inactive enantiomer, WIN 55,212-3. A second study examined the effect of WIN 55,212-2 on striatal-stimulation-evoked activity in the globus pallidus. Striatal stimulation produced a brief inhibition of neural activity in the globus pallidus. WIN 55,212-2, but not WIN 55,212-3, reversed the inhibition produced by striatal stimulation. The inhibitory effect of WIN 55,212-2 on the basal activity of neurons in the globus pallidus was apparent in the striatal stimulation study as well. These observations suggest that cannabinoids may produce two different effects on neural activity in the globus pallidus - inhibition of basal activity and a reversal of the inhibition produced by striatal stimulation.

749.6  
EFFECT OF INTRANIGRAL ADMINISTRATION OF CANNABINOIDS UPON ROTATIONAL BEHAVIOR: INTERACTION WITH THE DopAMINergic SYSTEM. 

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Cannabinoid receptors have been reported to be highly concentrated in brain areas involved in the control of movement in accordance with their well-known effects on motor behavior. One of the areas with the highest concentration of cannabinoid receptors is the substantia nigra, which is the output of the basal ganglia for movement production and therefore has been classically used for studying neurotransmitter function in rotational behavior. cannabinoid receptors in this structure have been shown to be on the striatognral and dopaminergic neurons. The aim of this work was to study the effects of cannabinoids on the rotational behavior induced by unilateral injection of dopamine agonists into the rat substantia nigra pars reticulata. cannabinoid compounds have little effect on rotation when injected intranigral. However, they reduced the contralateral rotational induction by the dopamine agonists acting mainly through D1 receptors. These results are in accordance with the opposite action of cannabinoid and D1 receptors on second messengers and neurotransmitter release from striatognral terminals.

750.1  
D1 Dopamine Receptors Influence Fos Immunoreactivity in the Globus Pallidus and Subthalamic Nucleus of Intact and 6-OHDA-Lesioned Rats. 


The globus pallidus (GP) and subthalamic nucleus (STN) are major nuclei of the basal ganglia. Studies of the dopaminergic physiology of these nuclei have typically emphasized the role of the D2 dopamine receptor. However, effects of D1 receptor agonists on neuronal activity and motor function have been reported, especially after nigrostriatal lesion. We have systematically investigated the effects of D1 and D2 dopamine receptor activation on the activity of the GP and STN in sham-lesioned and 6-hydroxydopamine (6-OHDA)-lesioned rats using immunostaining of brain sections for the immediate-early gene Fos. 4-5 wks post-ops, rats were drug-injected and perfused 2 hrs later. Brains were sectioned on 40um and stained with an anti-Fos antibody. In intact rats, the D1 agonist SKF 38393 (20 mg/kg) produced a five-fold potentiation of the GP Fos due to the D2 agonist quinpirole (0.5 mg/kg), while having no effect alone. In the STN of intact rats, only the combination of SKF 38393 and quinpirole produced significant Fos. In the lesioned hemispheres of 6-OHDA-lesioned rats, SKF 38393 increased the Fos immunostaining in both the GP and STN, while quinpirole increased Fos in the GP. SKF 38393 effects were increased in the GP and STN of 6-OHDA-lesioned rats were blocked completely by the D1 agonist SCH 23390 and unaffected by the D2 agonist eticlopride, confirming the specificity of the SKF 38393 effect. In the lesioned STN, SKF 38393 and quinpirole may be due to increased input from the STN. An excitatory action of dopamine agonists on the STN is unexpected given present basal gangla models, and may reflect an as yet novel demonstration of control of Fos expression by dopaminergic drugs in the STN and by D1 agonists in the GP.

750.2  

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It has been proposed that one of the functions of the basal ganglia is to act as a 'state integrator' of motor and limbic information. Although it is clear that there are multiple parallel pathways through the basal ganglia, it is not clear where, or indeed; these pathways converge. In the present tracing and immunocytochemical study of the descending projections of the reticulospinal (posterolateral-hypoglossal) GP was addressed. DAbel antergrade tracing revealed that medial GP and VP innervated largely subcortical and certain other areas. In zones of overlapping termination, the somata of individual neurons were apopled by GP and VP boutons. Single antergrade tracing with retrograde labeling reveals that neurons in the GP have been shown to project to GP and VP. In addition, the subthalamic dopamine neurons (e.g. tyrosine hydroxylase-immunonegative) were also profusely opposed GP or VP. However, the complex organization underlying these patterns of innervation are currently being re-examined by correlated light and electron microscopy. This analysis indicates that individual neurons in GP do indeed receive convergent synaptic input from GP and VP. We therefore propose that while the basal ganglia closely process information that is related to movement, in parallel, some convergence of information occurs at the level of individual neurons (funded by Wellcome Trust and MRC, U.K.)
EM MORPHOLOGY REVEALS THAT NEUROTENSIN IMMUNOREACTIVITY IS ELICITED IN DIFFERENT SUBPOPULATIONS OF STRIATAL AXONS FOLLOWING DOPAMINE D2 RECEPTOR ANTAGONIST AND RESERPINE ADMINISTRATION. D.S. Zahm*, E.S. Williams and B. Senger. Dept. of Anat. and Neurobiol., Saint Louis University. School of Medicine, St. Louis, Missouri 63104.

Perikaryal staining for neurotensin immunoreactivity (IR) is undetectable in normal rat striatum, but is elicited following administrations of dopamine D2-receptor antagonist and reserpine analogs that target perikarya and fibers (Neurosci., 46:3335, 1992; 57:649-660, *93 & 65:71-86, *95). Whether elicited by D2 antagonists, neurotensin, or NT-IR neurons have been shown to project to the globus pallidus and substantia nigra pars reticulata, and to coexpress the substantia nigra A1 complex (Soc. Neurosci. Abstr. 19:129, 1993 & Bog & Zahm, unpublished). Rats were given either two injections (2 mg/kg) of haloperidol or eticlopride at four and twenty-four hours prior to sacrifice or a single injection of reserpine (5 mg/kg) twenty-four hours prior to sacrifice and killed by perfusion with buffered aldehydes. Frontal sections were processed for NT-IR and flat embedded for transmission electron microscopy. A random sampling method and computer-assisted morphometric analysis revealed NT-IR-bouton profiles that are significantly smaller following reserpine than after haloperidol administration (p<0.001, Maze-Whitney U test). Fewer NT-IR synapticboutons were observed following haloperidol (1.6% vs 3.0%, p<0.012, paired t-test), probably due in part to the lesser probability of section planes encountering synapses in larger terminals. Synapses were almost exclusively symmetric axodendritic. The EM data are consistent with morphologically distinct subpopulations of striatal neurons that express NT.

Supported by USPHS NIH NS-23805 (NINDS).


Intrastriatal injections of quinolic acid (QA), an NMDA receptor agonist, produce a loss of specific projection neurons in the globus pallidus and substantia nigra, as well as the expression of c-Fos in the neostriatum found in Huntington's disease. We have previously shown that DNA strand breaks are present in striatal neurons in HD and after QA injections. DNA damage was detected between 6 and 10 hours after QA injections. Expression of met-enkephalin (ENK), substance P (SubP) and glutamic acid decarboxylase (GAD-87) was examined with immunohistochemistry in striatal projection areas before and after DNA damage is evident in the stratum. Male Sprague-Dawley rats (250-300 gms) received a unilateral injection of QA (60 nmol in 0.5 mL) or saline over 5 min. in the striatum. This procedure induces a massive loss of striatal efferent neurons 2 weeks later (Chetin et al. Exp. Neurol. 115:1992-2011). Rats were sacrificed 10 or 12 hours after surgery by transcardial perfusion with 4% paraformaldehyde under deep anesthesia. Sections were processed for immunohistochemistry with avidin-biotin-peroxidase and diamidinedehyde as the chromogen. None of the rats sacrificed 10 hrs after QA injection in the striatum showed conspicuous changes in ENK immunoreactivity (IR) in the ipsilateral globus pallidus (GP). In contrast, a marked decrease in ENK IR was already observed in some of the rats sacrificed 12 hrs after QA injection. In these same animals GAD 67 IR in the GP and SP IR in the entopeduncular nucleus were also decreased. Thus, intrastriatal QA induces a rapid decrease in GAD and neuropeptide expression in axons of striatal efferent neurons, which appears to coincide with the onset of detectable DNA damage in their cell bodies. Supp. by MH-44894 and F30-MH-10890.

VENTRAL MESENCEPHALIC NEURONS RETROGRADELY LABELLED WITH WGA-HRPpapGold OR FlUORO-GOLD FOLLOWING INJECTIONS IN THE SHELL, CORE AND ROSTRAL POLE OF THE NUCLEUS ACCUMBENS: IMMUNOHISTOCHEMICAL AND MORPHOMETRIC ANALYSIS Y. Tan, J.S. Bog*, E.S. Williams and D.S. Zahm. Dept. of Anatomy and Neurobiology, Saint Louis University School of Medicine, St. Louis, Missouri 63104.

The mesencephalic projection constitutes a morphologically and neurochemically heterogeneous population of axons. Ventral mesencephalic dopamine (DA) neurons exhibit several distinct types of somatodendritic morphology. Some studies have shown that certain morphological types are associated with topographically and morphologically specialized axon projections. In the present study, either WGA-HRPpapGold or Fluoro-Gold (FG) were used to retrogradely label neurons in the ventral mesencephalon by injection into the shell, core and rostral pole of the nucleus accumbens. Retrogradely labeled neurons were evaluated for calbindin immunoreactivity, perikaryon diameter and labeled dendrite length. Following all injections except those in medial shell, core and rostral pole immunoreactivity was colocalized in a subset of retrogradely labeled neurons. Mean perikaryon diameter of FG labeled neurons was significantly less following injections in the medial shell than after injections in the core and rostral pole. Likewise, the median width of FG-labeled dendrites was significantly less following injections in the medial shell than following injections in the core or rostral pole. One hundred neurons from each of three cases for each subterritory were evaluated with the aid of a camera lucida stage. The data are consistent with a small to moderate component of midbrain DA/catecholamine projections to core-rostral pole and a morphologically distinct population of DA neurons projecting to medial shell. NIH NS-23805 (NINDS).


Chronic treatment with classical neuroleptics affects a number of neurotransmitter systems in the basal ganglia. For example, we have shown that D1-receptor binding in the EP is enhanced after reserpine. Furthermore, increases in striatal PEK mRNA expression have been reported by several groups. However, the specific dopamine (DA) receptor subtype involved in the regulation of these protein and expression is of the present study was to examine the regulation of D1-receptor binding and PEK mRNA levels using selective dopamine antagonists, adult rats were injected twice daily for 21 days with distilled water, SCh-23390 (0.1mg/kg), Raclopride (0.1mg/kg) or both Raclopride and SCh-23390 (Nod/gp). Animals were killed 48 hrs after the last injection. Sections were processed for autoradiography and in situ hybridization studies. For receptor binding assays, sections were incubated with [125I]SCH-23382 (D1) or [125I]NQ-296 (D2). An oligonucleotide probe selective for PEK mRNA was used for in situ hybridization experiments. Autoradiographic analysis revealed that Raclopride slightly but significantly increased [125I]NQ-296 binding in the STR (10%), subthalamic nucleus (16%) and substantia nigra compacta (12%), whereas SCh-23390 significantly enhanced [125I]SCH-23390 binding in the EP (43%), STR (17%) and substantia nigra reticulata (32%). Striatal PEK mRNA levels were significantly elevated by Raclopride (16%), SCh-23390 (41%) and by the combination of Raclopride and SCh-23390 (62%). Two-way ANOVA of the PEK mRNA data revealed that Raclopride and SCh-23390 enhance PEK mRNA expression through separate mechanisms. Supported by grant 86318 and 91274 from the Swedish MRC.


C-Fos induction can occur as a consequence of synaptic activation at times when the cells are not actively dividing. C-Fos was induced in the hypothalamus, thalamus and cerebellum following injection of kainic acid into the striatum. The results indicate that there are functional connections between the medial prefrontal and the mesoaccumbens. The mesoaccumbens is essential to the organism's survival. The Accb may be divided into subterritories based on neurochemical, morphological and hodological properties. Given the importance of this structure, the distinction of distinct dopaminergic axon types is critical to determine whether the different connectivity patterns of Accb subterritories are functionally relevant. In order to address this question, 6-hydroxydopamine microneurotoxications were made in the Accb shell of male Sprague-Dawley rats. The lesion sites and lateral spread were evaluated using tyrosine hydroxylase immunohistochemistry and Fox expression was assessed immunohistochemically at several post lesion time points. Preliminary evidence of the material revealed a robust enhancement of Fox immunoreactivity bilaterally in the medial prefrontal, insular and piriform cortices, septum, Accb and medial caudate-putamen of lesioned animals as compared to ascorbate vehicle and saline controls. Control animals exhibited moderate numbers of Fox-IR cells in the same brain regions. Therefore, periventricular cells will be quantitated and statistically compared. Insofar as all of these areas receive mesencephalic dopamine input from ventral mesencephalic neurons within the projection field, the development of a novel immunohistochemical approach to the identification of dopaminergic axons is likely to provide an improved method for the quantitation of dopaminergic axons in lesioned and control animals. This is the first report showing that the dopamine neurons are disinhibited following focal Accb lesions through increased inhibition of midbrain dopamine neurons. Supported by USPHS NIH NS-23805 and NS-07254 (NINDS).

Methylphenidate (MPH) is a dopamine re-uptake blocker with effects on mesolimbic and mesostriatal dopamine (DA) systems. To identify neuronal subregions and neuronal phenotype effects in DA systems, in situ hybridization histochemistry was performed after acute (at 7X) or chronic ip treatment (twice daily, 4.7X) on saline, low (1.25 mg/kg) or high (12.5 mg/kg) MPH. mRNA labelling for D1 and D2 receptors and D4GTPase were measured in high-dose in the VTA and SN, and GADgpr, DA1 and DA2 receptors and peptide regions in the medial and lateral subregions in the STR. Locomotor activity, stereotypy and rearing were measured on day 7, 14, and 21. After drug treatment, all behaviors increased in the high-dose group (p's < 0.005) and there were no changes in mRNAs labelling. With chronic treatment, activity was highest in the low-density group (p = 0.0005), and effects were largest on day 1 (p's < 0.004). Stereotypy showed dose-dependent increases (p < 0.0001) with chronic treatment with all doses resulting in increased TH labelling in the VTA and SNC (p's < 0.05). High-dose treatment resulted in increased labelling for PPE in the STR (p < 0.01). Overall treatment was changed suggesting that this was not due to loss of DA terminals. Chronic MPH treatment alters gene expression in VTA and SNC DA neurons and STR efferent neurons which express PPE. Supported by Tourette Syndrome Association, HD26815 and HD19797.


The serotonin 5-HT2A receptor may be involved in the regulation of the opioid peptide dynorphin in the ventral striatum, as suggested by changes in preprodynorphin (ppdyn) mRNA after a lesion of the serotoninergic system. On the other hand, enkephalin synthesis does not seem to be affected by a serotonergic lesion as there is no change in preproenkephalin (ppenk) mRNA levels. We set out to investigate whether there is an anatomical basis for these different responses of opioid neurons. Double-labeling in situ hybridization experiments revealed a clear tendency to demonstrate 5-HT2A ppdn/pptpn mRNA in the same sections. 5-HT2A mRNA was visualized with a [35S]-labeled riboprobe, whereas a digoxigenin-labeled riboprobe was used to demonstrate ppdn/ppen mRNA. Opioid neurons were identified and overlying grains generated by the radioactive probe were counted with an IBAS image analysis system. Our results demonstrate that 5-HT2A mRNA is localized in both enkephalinsensitive and dynorphin-sensitive cells. In different subregions of the ventral striatum (medial shell, core, olfactory tubercle), 40-45% of the enkephalin neurons contain detectable levels of 5-HT2A mRNA. However, in the lateral shell the percentage of co-localization is significantly higher (60%) than in the other subregions. Our results further indicate that 35-45% of the dynorphin cell population in the core and medial/lateral shell synthesizes the 5-HT2A receptor. In the olfactory tubercle, there is a significantly higher degree of co-localization (65%) compared to the other ventral striatal regions. On the basis of these data, we would suggest that 5-HT2A receptor manipulation can directly affect the populations of both types of opioid output-neurons. The magnitude of the effect of 5-HT2A receptor manipulation might differ between subregions of the ventral striatum.

T50.12 CELLULAR LOCALIZATION OF D1 AND D2 Dopamine RECEPTOR mRNA IN THE ACCUMBENS/PALLIDAL AND ACCUMBENS-VENTRAL Tegmental AREA PATHWAYS IN THE RAT. C.F. Marin, D.F. González, M. P. W. Kalivas. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA, USA.

The involvement of the ventral striatum in mediating motor activity is thought to arise via afferent projections from the nucleus accumbens which in turn receives the dopaminergic input from the mesencephalic tegmentum. Two major families of dopaminergic neurons have been characterized in the central nervous system and designated D1 and D2 dopamine receptor subtypes. Using in situ hybridization methods combined with in situ hybridization, this study examined the postynaptic distribution of accumbal D1 and D2 dopamine receptors following microinjection (1 pmol) of Fluoro-Gold in the ventral pallidum (VP) and ventral tegmental area (VTA). The data indicated that the portion of retrogradely labeled neurons with Fluoro-Gold deposits in the VP that contained messenger RNA for D2 dopamine receptors was 19-37% in the shell, 19-36% in the core of the nucleus accumbens, whereas 40-67% and 50-73% were double-labeled for D1 and D2 receptor messenger mRNA in the shell and core subcompartments, respectively. Preliminary evidence also showed double-labeled neurons with Fluoro-Gold deposits in VTA for D1 receptor messenger RNA ranging from 64% to 100% along a caudal-rostral gradient. These data demonstrate that both D1 and D2 receptors, predominantly D2 receptors, exist in the pathway from the nucleus accumbens to ventral pallidum, suggesting D1 and/or D2 receptors on accumbal neurons may directly modulate neurotransmitter release in the ventral pallidum, alter the firing frequency of pallidal neurons, and subsequently affect locomotor activity. This study also reveals that most accumbal neurons projecting to VTA contain D1 receptor messenger RNA. This suggests that dopamine release may act on D1 receptors in the nucleus accumbens to regulate the activity of dopamine cells in the VTA.


Lithium is the most effective agent for the treatment of manic depressive disorder. The forebrain dopaminergic system, among others, is suggested to play a major role in acute mania. To identify the neuronal circuitry that is responsive to lithium, we mapped lithium induced Fos expression in the forebrain. Injections of lithium chloride induced Fos expression selectively in the "hippocampal district" of the accumbens, but not in rest of striatum. Numerous Fos positive neurons were noted in the hippocampal CA1, CA2 regions, entorhinal, prelith, infralimbic, agranular insular and pyriform cortex, but only a few Fos positive cells were noted in deep layers of many cortical areas. Fos reactive cells were restricted to the mediodorsal nucleus of the thalamus. Cells with Fos-Li were conspicuously absent in VTA & substantia nigra complex. The results suggest that the hippocampal district of accumbens & hippocampus and the high-dose regions that converge on this specific area of limbic striatum may mediate lithium induced benefits on acute & recurrent manic attacks. Supported by Smokeless Tobacco Research Council, NY & Department of Defense.


Recent studies have revealed that there are many similarities in the organization of the basal ganglia (BG) between reptiles, birds, and mammals. The intriguing question raises, therefore, whether this basic organization exists also in anamniotes. In amphibians, not only a dopaminergic cell group in the midbrain, but also distinct plexuses of DA fibers in the ventromedial (accumbens) and ventrolateral (striatum) wall of the telencephalic hemisphere have been identified. In contrast to amniotes, the midbrain DA cell group of amphibians is rostral continuous with DA cells in the dorsomedial part of the posterior tubercle (TPm), and does not extend caudal to the caudolateral nerve. It is also impossible to recognize separate A-9/A-10 cell groups, indicating a preferential signal in the development of the BG in amphibians. To get more insight into the organization of the DA telencephalic connections, combined tract-tracing/immunohistochemical techniques have been used. In the present study, Anuran postmortem brains (Rana rugosa, Xenopus laevis) were applied to the basal forebrain of anurans (Rana peronii, Xenopus laevis). As marker for DA cells, we have used tyrosine hydroxylase (TH) immunohistochemistry. Experiments involving the accumbens yielded some double labeled cells in the TPm and, more frequently, in the midbrain tegmentum. Involvement of the striatum, on the contrary, resulted in many double labeled neurons, both in the midbrain. Thus, the nucleus accumbens and the striatum of anurans receive topographically organized DA inputs which, however, display a preferential caudal-to-rostral, instead of a mediolateral-to-arrangement as demonstrated for amniotes. Supported by DGICYT PB93-0083.
750.15

ASTROCYTIC GLUTAMATE AND DOPAMINE RECEPTOR SUBTYPE CO-LOCALIZATION IN THE RAT SUBSTANIA NIGRA. A.Matsumoto, J.W. Prechtl, K.H. Thomas, J.A. Whittington. Department of Anatomy, Morehouse School of Medicine, Atlanta,GA,30310.

Abnormalities in CNS dopaminergic neurotransmission has been hypothesized to be related to actions of excitotoxic amino acids (EAA). There is increasing evidence that astrocytes may have a functional role in EAA-mediated brain injury and, as well, may modulate their phenotypic expression. Electrophysiological studies have indicated direct signaling between astrocytes and neurons suggesting the possibility of an astrocitary involvement in nigral degeneration and related disease states. We have previously localized glutamate and dopamine receptor subtypes within the rat substantia nigra (SNs). This study was designed to further investigate those receptor subtypes distributions and their relationship with astroglial in the pars compacta of the rat SNs. Using immunostaining techniques, we studied 2D3, GluR2/3, NMDAR1; and NMDAR2A/B receptor subtype expression, in the adult rat brain. Coimmunostaining with antibodies to glutamate receptor and the parvalbumin subunits and against the astrocyte marker, GFAP, were used in this study. FITC labeled GluR2/3, D2/3, NMDAR1 and NMDAR2A/B receptor immunoreactivity was observed in a heterogeneous pattern throughout the SN in close association with GFAP/Rhodamine labeled astrocytes. Our results suggest DA and glutamate receptor subtypes are co-localized in the cytoplasmic compartment of SN astrocytes.

SUPPORT: NIH grants S06GM08248 and 3G12RR03034.


750.16

APOMORPHINE TRANSIENTLY REDUCES SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE SUBSTANIA NIGRA IN 6-OHDA RATS. S.Gabrielsen, A.Naylor, S.Young. Department of Neurology, Oregon Health Science University, Portland, Oregon, 97222.

Substance P is a peptide which is contained in striatogniral neurons and present in high amounts in the SN. Lesioning and repeated drug treatment affect nigral substance P content; 6-OHDA lesioning reduces nigral substance P levels and daily L-DOPA treatment reverses this decrease. In the current study, we wished to determine if acute drug treatment also differentially affects substance P content in the lesioned SN. Eight rats with 6-OHDA lesions received 8 daily doses of apomorphine (0.1 mg/kg), a regimen which produced behavioral sensitization. The next day, four 3.2 mg/kg apomorphine doses were administered at 2 h intervals, a regimen which produced tolerance. Four rats were perfused 1-2 h later; the other four were perfused 1 week later. The density of substance P-like immunoreactivity (SPLI) in the SN on the 6-OHDA and normal side were compared by image analysis. In the acutely treated rats, the SPLI in the lesioned SN was reduced relative to the other side, whereas the rats untreated for 1 week after drug treatment exhibited symmetric staining. These findings suggest that administration of a dopamine agonist temporally reduces substance P levels in the SN ipsilateral to a 6-OHDA lesion, possibly reflecting an increase in striatogniral neurotransmission from the dopamine-depleted striatum. The observation that acute and chronic treatment produce different behavioral effects and corresponding changes in nigral substance P levels suggests that substance P may have a modulatory role in affecting dopaminergic response in animals with nigrostriatal lesions.

750.17

FUNCTIONALLY AND ANATOMICALLY DISTINCT DOPAMINE SYSTEMS IN THE MEDIAL PREFRONTAL CORTEX: AN APPRAISAL. A.Y. Deutsch. Departments of Psychiatry and Pharmacology, New York University School of Medicine, New Haven, CT and Veterans Administration Medical Center, West Haven, CT 06516.

Several cortical regions in the rat receive dopamine (DA) innervations, the most prominent of these being the medial prefrontal cortex (PFC). DA projections to the PFC are derived from cells in the ventral tegmental area (VTA). The mesoprefrontal cortical DA innervation has been considered to be a functionally homogeneous DA innervation. However, the DA innervation of the rodent PFC appears to be homologous to several spatially distinct in the primate that have different functional attributes. We have recently observed that several DA innervations of the PFC in the rat can be autonomic, biochemically, and molecularly distinguished, and that these different DA innervations respond different to different challenges, including mild stressors and antipsychotic drugs.

We propose that there are several different DA innervations of the PFC in the rat that in part in parietoarchitectonically-defined regions, that these different DA innervations of PFC regions are derived DA neurons in different subnuclei of the VTA, and that various challenges activate both a DA innervation of a particular part of the PFC (e.g., the infralimbic or dorsal prelimbic cortex) and concomitantly a particular cluster of midbrain DA neurons. Supported in part by MH-45124, the NPF Center at Yale, and the VA Schizophrenia and PTSD Centers.

CEREBELLUM: PHYSIOLOGY

751.1


The gain change hypothesis proposed by Eber and Bledsoe states that the climbing fiber input to a Purkinje cell (P-cell) produces a short-lasting enhancement of its responsiveness to mossy fiber inputs. We examined the simple spike (SS) activity patterns that followed the complex spike (CS) of flocular P-cells in ketamine-acetazolamide anesthetized rabbits. We recorded from 43 P-cells whose CSs were modulated by optokinetic stimulation (OKS). Three conditions were investigated: spontaneous activity in light and dark and constant speed OKS. Data analysis included CS-SS cross-correlation and SS auto-correlation. P-cells were assigned to one of three categories: pure pauser, pure facilitator (PF), or pause-reduction (PR). To be categorized as a PF (or a PR) cell, the average SS activity during at least one 20 sec period within the first 50 msec after the end of the CS was greater than 1.2 times the average SS activity during the 100 msec prior to the CS. Of the 43 P-cells, 26 (60%) were PF cells, 9 (21%) were PR cells, and 8 (19%) were PF cells. The categorization distributions were consistent with the in the awake rabbit (Simpson, Wylie and De Zeeuw, submitted), the percentage of P-cells showing enhancement of responsiveness to mossy fiber inputs doubled and the SS rhythmically markedly increased. Thus, ketamine-acetazolamide anesthesia produces enhancement and SS rhythmically. (Supported by NS-13742).

751.2

ANALYSIS OF CA2+ SPIKE AUTORHYTHMICITY IN CEREBELLUM PURKINJE CELLS. Y. Eitan, and Y. Grossman. Department of Physiology, Faculty of health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

Spontaneous Ca2+ spike activity in cerebellar Purkinje cells is characterized by a regular alternation between bursting and quiescence periods and a propensity for firing in doublets. We studied the underlying mechanisms of this behavior in guinea pig cerebellar slices. Slices were constantly perfused with 1mM KCl Ringer’s solution equilibrated with 95% O2, 5% CO2 at 30°C. Spontaneous Ca2+ spike currents were recorded by using a macropatch clamp technique before and after blocking sodium currents with 0.5mM TTX. TTX significantly increased active period duration to 149.3±44.9% (mean±SD, n=14), silent period duration to 142.1±53.8% and the mean inter spike interval (ISI) to 150.9±63.4% but did not change doublet spiking. Application of CNQX (10mM) and Bicuculline (20mM) did not mimic TTX effects. Heating to 33°C enhanced doublet firing, while cooling to 28°C markedly increased mean ISI and almost abolished doublet firing. Inhibiting [Ca2+]i to 1-2 nmol, addition of 4-AP (10mM), or TEA (0.1mM) greatly increased doublet spiking and could counteract the effect of cooling. Whereas, reducing [Ca2+]i to 3nmol, like cooling, almost abolished doublet spiking. The ISI in doublets changed gradually by these manipulations from few ms to approximately 40 ms. These results indicate a) Invasion of somatic Na+ spikes may affect dendritic Ca2+ spike autorhythmicity. b) Existence of a highly sensitive K+ current which reduces doublet spiking. c) The range of modulation of ISI within doublets can not be attributed to spike propagation between two zones of the dendritic tree, and therefore support the notion of a single site for doublet generation.
CEREBELLUM: PHYSIOLOGY

WEDNESDAY PM

751.3

A monkey was trained to move the arm about 1 sec. following an auditory stimulus. In the paramedian lobule of the cerebellum we recorded rhythmic bursting of thin, small spikes at the frequency of 14-16 Hz. The profile of local field potentials and oscillations at this same frequency was maximal in the granular layer and Purkinje cell activity recorded in adjacent layers was not modulated at that frequency. So far we have been able to establish the following properties of this oscillatory process: 1. It is very much decreased during reduced state of vigilance and after administration of harmaline. 2. It is reduced and disorganized some 150-250 ms after the external cue, resumes 200-250 ms later and stops abruptly at about movement onset; 3. the oscillations are enhanced in the 400-500 ms period preceding movement onset. This is not observed with spontaneous movement; 4. olivo-cerebellar activity tends to increase 150-250 ms after the external cue at a time where the rhythmic process appears to be reset. More investigations are needed to establish the functional role of this oscillatory process which could be involved in the precise timing of movement onset in response to external events. Supported by MRC of Canada.

751.5
FUNCTIONAL ORGANIZATION OF THE RAT CEREBELLAR CORTEX ELUCIDATED IN VIVO USING THE PHOTOBLEACHING, Neutral Red, Optical Imaging Technique. C. Hansen, Q.G. Fu, G. Chen, T.J. Ebner. Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

The highly regular cellular and circuitry of the cerebellar cortex and itsafferent and efferent systems are organized around several spatial plans. Optical imaging is one approach used to record the patterns of activity in the cerebellar cortex. In this study, we used principal component analysis (PCA) to extract spatial patterns of activity from optical images of rat cerebellar cortex under conditions of peripheral or surface stimulation. Optical signals from stimulus-evoked fluorescence changes in cerebellar cortical area Crus II were recorded with a CCD camera in anesthetized rats (ketamine/xylazine). The tissue was superfused with Tyrode solution at 37°C, using cannula. Ongoing and synchronized, rhythmic discharge of neuromuscular junctions was accompanied with reports suggesting that increases in circulating levels of E is facilitated, rapidly, alternating stimulation of the limb, and the re-stimulation of olivary oscillations, as providing the frequency template for rapid limb movement (Limas, 1992). For this purpose, stimulus trains from the whisker receptive area of the rDA2 were recorded simultaneously with cells from corresponding positions in the anteroventral lateral (AVL) nucleus of the caudal, bushy and caudal, subparietal nucleus (NB, Lades, Denver, TX). Activity from these three CNS sites could be monitored (Spectral Scientific, Dallas) during active whisking 24 h following injection of E2 (20 mg/kg, ip, for 3 days). Results show that an active period of active whisking were accompanied by rhythmic rDA2 discharge at roughly the same frequency as the average frequency of the movement. In contrast, the PV and SpV demonstrate minimal oscillatory activity during whisking behavior. E1 enhanced the oscillatory discharge of the rDA2 both by eliciting the number of synchronized, oscillating cells (by ~7%) and by enhancing the amplitude of the oscillations (by ~30%). The influence of the oscillations on the underlying activity is achieved by facilitation of oscillatory activity as either the PV or the SpV (15-30 cells recorded per CNS area). Preliminary videoanalysis of concurrent whisking activity, filmed during the recording paradigm, suggests that the rhythmic neuronal activity enhanced by E1 was correlated with highly rhythmic whisking. E2 administration provoked whisking at a consistent, frequent interval, in a highly variable whisking frequency observed under control, diuresis conditions (5-10 Hz). These results suggest the facilitatory effect of E2 on rhythmic, synchronized rDA2 activity may underlie, at least in part, its observed behavioral effect in female (Rhythmic whisking movement supported by USAF 95-5-1336 to SSS and ONR N00014-95-1-0346, NS3722 to RCC).

751.6
OPTICAL IMAGING OF THE PARASAGITTAL ORGANIZATION IN THE RAT CEREBELLUM CORRELATE TO THE NEURAL CIRCUITRY. J.M. Division of Biology, Caltech, Pasadena, CA. 751.7

RELATIONS BETWEEN PURKINJE CELL SIMPLE SPIKE ACTIVITY AND MOVEMENT VELOCITY AND POSITION IN MONKEYS USING A NOVEL, TWO-DIMENSIONAL TRACKING TASK. J. D. Goller and J. J. Ebner. Graduate Program in Neuroscience and Dept. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, MN 55455.

Previous electrophysiological studies of cerebellar Purkinje cells in our laboratory (Fu et al., 1994) found a significant correlation of simple spike discharge with movement parameters such as direction, frequency, and target position. However, the variance in cell discharge accounted for by these parameters was moderate, suggesting that other variables are needed to describe the motor output of these cells. The present study employed a novel, two-dimensional visuomotor task that required monkeys to track moving targets at 5 different velocities, reaching from a centrally located start position to endpoints in opposite directions. The velocity at which the moving targets were displayed was then changed in 2 conditions, and the peak velocities ranged from 2 to 6 cm/sec. Single trial analyses of the Purkinje cell simple spike discharge utilized a multiple regression model. The skin temperature of the cell discharge was sensitive to multiple factors for hand position, velocity, and acceleration. The overall R2 for all cells was greater than that obtained in the earlier study (36%). The topography of the movement fields was best represented by the movement patterns, with each parameter of the cells were quantified by calculating partial R2 values. Velocity information contributed more to the overall strength of the model than did position or acceleration information in 19 of 30 cells. In 11 of 30 cells, position information contributed more than that about velocity or acceleration. These preliminary findings indicate that movement velocity and position information correlates of simple spike activity in the primate cerebellum. Supported by NIH grants NS 18338 and NS 31520.

751.8
ESTRADIOL FACILITATES SYNCHRONIZED OLIVARY OSCILLATIONS DURING WHISKER MOVEMENTS IN THE RABBIT. J.M. Hansen, J. J. Ebner, J. J. Ebner, and S. Smith, Dept. of Anatomy and Neurobiology, MCFH, Lawrence Univ., Appleton, WI.

Ongoing studies from this lab have demonstrated that iatreatal (E2) administration at physiological doses or just prior to estrus in the rabbit, enhances synchronized, rhythmic discharge of neurons within the dorsal raphe nucleus (DRN) (Hansen, et al., 1997). We have reported (Hansen, J.J., personal communication) that this effect is consistent with reports suggesting that increases in circulating levels of E2 is facilitated, rapidly, alternating stimulation of the limb, and the re-stimulation of olivary oscillations, as providing the frequency template for rapid limb movement (Limas, 1992). For this purpose, stimulus trains from the whisker receptive area of the rDA2 were recorded simultaneously with cells from corresponding positions in the anteroventral lateral (AVL) nucleus of the caudal, bushy and caudal, subparietal nucleus (NB, Lades, Denver, TX). Activity from these three CNS sites could be monitored (Spectral Scientific, Dallas) during active whisking 24 h following injection of E2 (20 mg/kg, ip, for 3 days). Results show that an active period of active whisking were accompanied by rhythmic rDA2 discharge at roughly the same frequency as the average frequency of the movement. In contrast, the PV and SpV demonstrate minimal oscillatory activity during whisking behavior. E1 enhanced the oscillatory discharge of the rDA2 both by eliciting the number of synchronized, oscillating cells (by ~7%) and by enhancing the amplitude of the oscillations (by ~30%). The influence of the oscillations on the underlying activity is achieved by facilitation of oscillatory activity as either the PV or the SpV (15-30 cells recorded per CNS area). Preliminary videoanalysis of concurrent whisking activity, filmed during the recording paradigm, suggests that the rhythmic neuronal activity enhanced by E1 was correlated with highly rhythmic whisking. E2 administration provoked whisking at a consistent, frequent interval, in a highly variable whisking frequency observed under control, diuresis conditions (5-10 Hz). These results suggest the facilitatory effect of E2 on rhythmic, synchronized rDA2 activity may underlie, at least in part, its observed behavioral effect in female (Rhythmic whisking movement supported by USAF 95-5-1336 to SSS and ONR N00014-95-1-0346, NS3722 to RCC).
CEREBELLUM:

Recent data showed that dorsal spinocerebellar (DSC) tract activity may encode the direction of hindfoot displacements as well as relative foot position (Bosco & Poppele, J. Neurophysiol. 70:863,1993). It is not clear, though, if these two signals are temporally segregated, or if they overlap in time.

We characterized the time course of DSC directional tuning using a typical center-out paradigm, passively displacing the cat's hindfoot 2 cm in eight different directions in the sagittal plane. Unit activity recorded in each position was significantly modulated by movement direction for 5-s following displacement, reflecting a broad directional cosine tuning.

In another experiment, the cat's foot was placed in several positions throughout the sagittal workspace, each position being approached from 3-4 directions in separate trials. For 60% of the cells, mean firing rates over 8 s were significantly correlated with the position of the foot relative to the hip. However, only 30% of the cells showed significant modulation with position for the first 2 s following displacement. The residual modulation was correlated with movement direction according to a directional cosine model. When this modulation component was subtracted from the total modulation over 8 s, the overall correlation of activity with foot position improved and became significant in 75% of the cells.

The results suggest DSC activity represents a combination of two concurrent signals: one related to the current foot position and the other to the direction of prior movement. The latter persists for several seconds after the movement.

Supported by a grant from the NIH (NS21143).

VESTIBULAR SYSTEM: VESTIBULAR NUCLEI


The chick tectal nucleus is a vestibular nucleus in the brain whose principal cells (PCs) undergo major developmental changes in structure and membrane properties at critical embryonic and hatching ages. During that period, there is evidence to support that changes in membrane excitability are correlated with the outgrowth of dendrites which start growing at 13 embryonic days (E13), and then double in length during the week between E15 and 1-2 days after hatching (N.Y. Acad. Sci. Abst., 1995). Using intracellular recording in brain slices, we have focused on the membrane properties of PCs to injected depolarizing current pulses of 400 msec duration with intensities ranging from 0.5 to 1.0 nA. At E13, all of the investigated cells (n = 4) responded with a single spike to 1.0 nA of depolarizing current. At E15-16, similar currents produced either a single spike (n = 5) or in 6 other cells, the depolarization induced the firing of several action potentials (APs). This firing was irregular in nature, amplitude and time interval between APs and occurred with an average discharge rate of 32 APs/sec. At H1-2, PCs responded the same depolarizing current by repetitive firing discharge rate, 68 APs/sec of APs with regularly-spaced intervals between the APs (n = 7). These observations indicate that by hatching PCs can respond to rapidly occurring synaptic events. Accordingly, parallel to the extensive outgrowth of dendrites in newborns, which could subserve an increasing number of synaptic inputs, PCs begin to acquire membrane properties which allow the cells to follow vestibular stimuli. Supported by NIH grant R01-DCC0970.

2. NEURAL CORRELATES OF VISUAL POSTURAL REFLEXES AND NEGATIVE PHOTOTOXIS IN LAMPERY. F. Jolicoeur, T. C. Delaunois, U. O. Orlovsky and S. Grillner. Dept of Neuroscience, Karolinska Institutet, Stockholm, SWEDEN

Eye illumination in lampry evokes both negative phototaxis (NP), and a roll tilt towards the light (dorsal light response - DLR). DLR can elicit the stimulus with 30,000,000-40,000,000. Evidence for the existence of a new, visual reflex was found in lampry. The neuronal correlate of DLR was investigated with in vivo recordings from retinolateral neurons (RLNs), which form the main descending system for roll control in lamprey. Optic stimulation gave rise to a depolarization of the vestibular response in lampry RLNs, which indicates that the roll control system was reconfigured to stabilize a tilted orientation. The presentation was largely due to increased excitability in pre-vestibular interneurons. To elucidate which intranuclear pathways mediate DLR and NP, behavioural experiments were performed on chronically lesioned animals. Optic nerve fibres in lampry project bilaterally to tectum, pretectum and thalamus ("lateral geniculate nucleus", LGN). These areas project virtually bilaterally to RSNs, commissural fibres first course ventrally and cross the midline in the basilar plate. Ablation of the tectum, Pretectum or NP, after illumination of the eye, the animal rolled several times around its longitudinal axis, and performed repeated yaw turns away from light. This could be explained by a general disinhibition of reticular nuclei after tectectomy. The same picture was seen after transection of the mesencephalic ventral commissure, which indicates that the tecto-reticular inhibition to a large extent is through crossing fibres. After unilateral ablation of the pretectum, which presumably also may damage some LGN cells and axons, both DLR and NP could still be evoked from the ipsilateral eye, contralateral visual responses were as a rule absent. Transection of the posterior commissure, which connects left and right pretectum, did not influence NP, nor DLR. Transection of the caudal mesencephalic ventral commissure saved DLR, whereas NP was replaced with positive phototaxis. Cells in commissural pretectum or LGN thus appear to play a major role both for NP and DLR, with fibres responsible for NP presumably crossing the midline at a homologous level.


The lateral vestibulospinal tract (LVST) provides a pathway through which the vestibuloneuraxons afferents can affect head stabilization and movement. Individual axons of LVST neurons were intracellularly recorded to determine their short-latency input from the labyrinth and labeled using biocytin. Main morphological features examined are: axon trajectory in brainstem and cervical spinal cord, and termination pattern and collateral distribution in the cervical spinal cord; in several cases the axon's cell body was recovered in the vestibular nucleus. Antidromically identified lumbar-projecting LVST axons often travel in the ventrolateral funiculus, but can course in the lateral funiculus along the cervical cord. In 10% of these axons provide a collateral input to the upper cervical ventral horn; the few axons that could be followed more posteriorly passed with the lateral funiculal junction with other pathways. Lumbar-projecting LVST axons thus appear to provide a direct channel to the hindlimbs. LVST axons terminating in the upper cervical segments often enter the spinal cord in the ventral funiculus, but can course from the lateral to the ventral funiculi. The termination pattern of these axons depends on the axon's funicular location; extensive synaptic input is provided to ventromedial nuclei IX and X, and laminae VIII and VII, the targeting primarily the motoneuronal cell groups. In some cases an axon travels and terminates like other LVST axons, but then changes funicular pathway and branches in a manner more characteristic of medial vestibulospinal tract cells. (Supported by PHS NINDS NS27050).


We examined the noradrenergic pathway from locus coeruleus (LC) to the vestibular nuclei (VN) in rats, rabbits, and monkeys. Noradrenergic projections were localized immunohistochemically with antibodies raised against catecholamine biosynthetic enzymes. The density of noradrenergic innervation was quantified. This analysis revealed a differential distribution of noradrenergic projections throughout VN. In all three species, innervation is highest in the lateral (LVN) and superior (SVN) VN. In rats, Detector neurons in LVN receive the densest projections. In monkeys, highest levels are seen in lateral portions of SVN, SVN, and medial-central portions of prepositus hypoglossi and MVN, and to a lesser extent in group VN. In rabbits, the highest innervation density was observed in SVN and SVN. In all species, innervation is highest in the lateral (LVN) and superior (SVN) VN. In rats, Detectors neurons in LVN receive the densest projections. In monkeys, highest levels are seen in lateral portions of SVN, SVN, and medial-central portions of prepositus hypoglossi and MVN, and to a lesser extent in group VN. In rabbits, the highest innervation density was observed in SVN and SVN. In all species, innervation is highest in the lateral (LVN) and superior (SVN) VN. In rats, Detectors neurons in LVN receive the densest projections. In monkeys, highest levels are seen in lateral portions of SVN, SVN, and medial-central portions of prepositus hypoglossi and MVN, and to a lesser extent in group VN. In rabbits, the highest innervation density was observed in SVN and SVN.

First, the neurotoxin 5-chloro-N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), was used to selectively block noradrenergic projections in rats. DSP-4 treatment greatly reduced noradrenergic innervation of VN. Second, retrograde tracing studies in the VN of rats (fluoroGold, rhodamine-dextran) and rabbits (HRP) indicated that the pathway originates only from the ipsilateral, ventral portion of caudal LC, subcoeruleus and A4. These results suggest that LC activity may play a role in modulating vestibular reflexes by direct action in VN and may thereby offer an anatomical substrate for increased vestibular performance during states of heightened arousal.
752.5 INDUCTION OF IMMEDIATE EARLY GENES DURING VESTIBULAR COMPENSATION. C.S. Darlington*, P.F. Smith, M. Dragunow* Dept. of Pharmacology, Univ. Qeensland, Deakin, N.2. VESTIBULAR compensation is a process of CNS plasticity that occurs after unilateral deafferentation of the vestibular nerve. Despite the severe ocular motor and postural symptoms that develop immediately following the lesion, many of these symptoms disappear within 2-3 days; this compensation process is correlated with a return of resting activity to type 1 neurons in the medial vestibular nucleus of the affected side. The effects of vestibular compensation on any of the most prominent symptoms, e.g., the effect on the latency (elimination; Post-operative 24 hours). On single-unit recordings of the whole eye movements, we found that c-fos expression with post-operative 24 hours persisted in the lateral side only. It may be that c-fos is induced at another time post-UL, e.g. 24 hours. The induction of c-fos is sometimes associated with cell death, however analysis of the contralateral MVL at 10 and 50 post-UL indicated no signs of apoptosis. At present, the significance of these results is unclear.

752.6 EFFECTS OF ELECTRICAL STIMULATION ON VOR AND C-FOS LIKE PROTEIN EXPRESSION OF THE MEDIAL VESTIBULAR NUCLEI FOLLOWING UNILATERAL LABYRINTHECTOMY IN RATS. B. E. Plaisant*, M.S. Kim, Y.S. Lee, O.J. Kim, Department of Physiology, Wake Forest University School of Medicine & Medical Research Center, Winston-Salem, NC 27103. The effects of electrical stimulation on vestibulospinal reflex (VOR) and c-fos like protein expression of the medial vestibular nuclei were investigated for 72 hours following unilateral labyrinthectomy (ULX). Electrical stimulation with 1 ms pulse and 100 Hz was applied to the lesioned vestibular system for 6 hours per day. ULX caused spontaneous nystagmus with fast phase to the intact side, whose frequency was 3-9°/0.2 beats/sec (M±SE) just after ULX but disappeared by postoperative 48 hours. On single-unit recordings of the whole eye movements, we found that c-fos expression with fast phase to the intact side persisted by rotation toward intact side, but the eye movement induced by vestibular stimulation toward the lesioned side recovered normal pattern by 24 hours at 0.1 Hz rotation, by 6 hours at 0.2 Hz. Directional preponderance which represents the symmetry of bilateral vestibular functions showed less than 20% on 48 hours, which implies the recovery of symmetry in bilateral vestibular functions. In electrical stimulation group, spontaneous nystagmus disappeared by 24 hours, and directional preponderance of VOR was less than 20% by 24 hours. On immunohistochemistry of the medial vestibular nuclei the number of c-fos like protein expression was 9.4±0.4 in each nucleus before ULX, but the number increased to 81.5±3.3 in the lesioned side and 298±2.0 in the intact side on 24 hours. On postoperative 24 hours the number in the lesioned side was 56.8±1.8 and in the intact side was 7.0±3.0. However, 48 hours after ULX, c-fos like protein was expressed equally in both nuclei. By electrical stimulation the number of c-fos like protein expression was symmetrical in both nuclei 24 hours after ULX. These results suggest that electrical stimulation ameliorates the recovery of VOR following ULX by modulation of the bilateral vestibular nuclear activity. Supported by MPPIC sponsored by KOSEF and KOSEF (951-0702027-2).

752.7 SYNDACTYL CONNECTIONS OF THE VESTIBULAR COMMENSAL PATHWAY IN THE CAT. F.A. Chaz and J.P. Forss. Department of Anatomy. Medical College of Virginia, Richmond, Virginia 23298. Vestibular commissural connections are believed to be mainly inhibitory in nature and are thought to provide some degree of cross-over reflexes, the enhancement of vestibular responses, and recovery of function from peripheral lesions of the VIIIth nerve. In the present study, injections of biocytin were made in to the medial vestibular nucleus of adult cats to anterogradely label commissural synaptic endings in the contralateral vestibular complex. By light microscopy, biocytin-labelled commissural axons were found to terminate ipsilaterally from the injection site, crossing the midline to the MLF, and forming terminal arborizations in homotopic regions of the contralateral medial vestibular nucleus. By electron microscopy, labelled commissural synaptic endings contained numerous small mitochondria and were classified on the basis of their content of spherical (64.1%), pleomorphic (31.2%) or flattened (4.7%) synaptic vesicles. Cross-sectional area of vestibular commissural endings ranged from 0.30 to 4.80 μm², with a mean of 1.35 ± 0.79 μm². Vestibular commissural synaptic endings were associated predominantly with distal dendrites, but were also seen in association with proximal dendrites and less frequently with somata and spine-like appendages. Post synaptic diameters ranged from 0.31 to 9.34 μm² with a mean of 3.36 ± 1.96 μm². Quantitative analysis of the soma-dendritic distribution revealed that 81.3% of endings terminated on distal dendrites, while 12.4% and 5.1% of endings terminated on proximal dendrites and somata, respectively. Only 1.1% of endings were found to terminate on spine-like processes. In most instances, each vestibular commissural synaptic ending established synaptic contact with only one postsynaptic profile. However, multiple synaptic endings arose from single commissal axons and contacted the same postsynaptic process. The results of this study suggest that the vestibular commissural pathway in the cat, like the vestibulospinal reflex pathway, is comprised of both excitatory and inhibitory components. Supported by USBF Research Grant 810291.

752.8 DISTRIBUTION OF THE CALCIUM-BINDING PROTEINS IN THE VESTIBULAR NUCLEI OF THE GUINEA PIG. W. Saunder, L. Pac, P. Metters, N. Garris, E. Godau and F. Pocket. Lab. Histologie, U.L.B., 1070 Brussels, Belgium. and Lab. of Neurosciences, Univ. of Mons-Hainaut, 7000 Mons, Belgium. In this study, we have undertaken a detailed mapping of calbindin, calbindin D2B, calretinin and parvalbumin in the vestibular nuclei of the guinea pig. Ten 14 μm transverse paraffin sections, either stained by cresyl violet, either immunostained (polyclonal antibodies) using peroxidase-antiperoxidase procedure and diaminobenzidine were analysed.

No neurons were parvalbumin positive in the superior vestibular nucleus, 80% of the neurons were calbindin positive and were uniformly distributed. 30% of the neurons were calretinin positive without any particular spatial distribution. The 10% of the neurons were calbindin positive were preferentially located in the dorsolateral part of the nucleus. In the medial vestibular nucleus, 70% of the neurons were calbindin positive and uniformly distributed. 32% were calretinin positive. 9% were calbindin positive and located along the medial side of the nucleus. In the lateral vestibular nucleus, 9% of the neurons were calretinin positive. Medium and small sized neurons were equally labelled. 31% were calbindin positive. Among them, 35% belonged to the giant or Dehers neurons. 3% which were calbindin positive were of small size. In the descending vestibular nucleus, 90% of the neurons were calbindin positive. 14% were calretinin positive. 10% were calbindin positive.

We conclude that, in vestibular neurons, calmodulin is highly expressed, calretinin and calbindin are less abundant and parvalbumin is absent.

752.9 SPATIOTEMPORAL PROPERTIES OF CENTRAL OTOCONE NEURONS IN DEVELOPING RATS. C.H. Lai and Y.S. Chan (SPON: The Hong Kong Society of Neurosciences). Department of Physiology, Faculty of Medicine, The University of Hong Kong. Saunders Road, Hong Kong. To examine the postnatal maturation of the spatiotemporal properties of vestibular afferent neurons, extracellular activities were recorded from the lateral and descending vestibular nuclei of decerebrate rats (7-21 days) and adult during off-vertical axis rotations (OVAR). The persistence of these extracellular discharges was examined in the sampled population decreased with age: 50% at 7 days of age and 27% in adult. Both the best response orientations of the narrowly tuned units and the preferred orientations of the broadly tuned units were distributed predominately in the transverse direction in 7-day old rats but were found in all directions on the plane of rotation in older rats. There was also a significant increase in the response gain of these neurons in older rats. Our results suggest that a coordinate frame of head positions with respect to gravity progressively coordinates within the vestibular nucleus in the course of postnatal development.

752.10 FUNCTIONAL CHARACTERISTICS OF ISPILATURAL AND CONTRALATRAL INPUTS TO CENTRAL OTOCONE NEURONS OF CATS AFTER ACUTE UNILATERAL LABYRINTHECTOMY. Y.S. Chan*. Department of Physiology, Faculty of Medicine, The University of Hong Kong. Sassoan Road, Hong Kong. To determine the nature of the ipsilateral and contralateral otoconial inputs, extracellular activities of vestibular neurons on both the lesioned and labyrint-hint intact sides were examined during off-vertical axis rotations (OVAR: 17°/sec, 15°/sec, 15°/sec, 15°/sec, 15°/sec, 15°/sec, 15°/sec, 15°/sec). The response ratio of the sensitivity of each neuron to OVAR in the CW and CCW directions was used to evaluate the spatiotemporal properties of the neurons. Those with symmetric and velocity-stable 8 were grouped as narrowly spatiotemporal-tuned neurons while those with asymmetric and velocity-variable 8 were grouped as broadly spatiotemporal-tuned neurons. More broadly tuned neurons were found in HL cats (a higher proportion on the lesioned side than on the intact side) than in control cats. This suggests that the more prominent broadly tuned responses in HL cats are normally masked by inputs from the contralateral side. On both sides, the directional preference of the broadly tuned neurons and the best response orientations to both of them were strongly located in the lesioned side. Thus, otoconial inputs from the two sides contribute to determine the spatiotemporal properties of the vestibular neural neurons in coding head orientations. (Supported by H.K. Research Grants Council.)

Responses arising from the vestibular nuclei encode the direction of linear accelerations at approximately 20°/sec. in rostral-caudal direction. These responses were elicited by single sinusoidal rotation (angular acceleration) about an ankle-tipped 20° from the vertical. Approximately 50% of neurons responded to angular acceleration, the remainder responded to linear acceleration. There were no differences in the mean firing rate of responses to linear and angular acceleration. The responses were elicited by rotations of 5.0° or 10.0°/sec., and 3.5° or 7.0°/sec. in the horizontal plane. Afferents optimally encoding all directions of the horizontal plane existed in roughly comparable numbers, especially if considering inputs from both labyrinthine. Yet studies of central vestibular neurons located in the lateral and inferior vestibular nuclei, as well as neurons located in the pontomedullary reticular formation, showed a preponderance of neurons responding more vigorously to roll (lateral tilt) than to pitch (fore- aft tilt).

Guided by observations that lesions that include the medial vestibular nucleus (MVN) abolishes (pitch) vestibulo-sympathetic reflexes, we examined the responses of neurons in the MVN and adjacent inferior vestibular nucleus (IVN) to 7 de cerebrate cats. Responses attributed to activation of otolith (utricular) receptors were recorded from constant velocity rotation in the animal's horizontal plane about an axis tipped 20° from the vertical (changing linear acceleration). Of 47 treated neurons, 28 were recorded from this stimulus. The 28 responded best when nose down or nose up. 2 responded best when one ear was down, and the other 1 responded best at intermediate positions near the planes of the vertical canals.

Many of these responsive neurons were also tested for horizontal canal input using sinusoidal rotation (angular acceleration) about an earth-vertical axis. 2545 neurons were modulated by this stimulus, with response dynamics consistent with canal input. In 41 neurons tested, 16 responded to both stimuli, suggesting convergence between utricular and horizontal semicircular canal afferents. These results suggest that in addition to its well-known input from the horizontal semicircular canals, the MVN may serve as an important relay for pitch linear acceleration stimuli. Supported by NIH Grants NS24930 and DC00693.

752.12 CALCIUM-DEPENDENT CONTROL OF SIGNAL TRANSFORMATIONS IN MEDIAL VESTIBULAR NUCLEUS NEURONS. Juan de la Coz, P.C. Dept. of Physiology and Biophysics, University of California, San Francisco CA 94143.

A combination of mechanisms at the cellular and network levels determine how head movement signals are transformed into the appropriate oculomotor commands. To investigate the cellular control of signal transformations in vestibulo-cerebellar pathways, we have assessed the effects of blocking calcium channels on the gain and dynamics of spike generation in Medial Vestibular Nucleus (MVN) neurons. MVN neurons recorded in avian brain stem were injected with intracellular currents to measure spike generation gain (slope of the relationship between mean firing rate and input current amplitude) and dynamics (timecourse of instantaneous firing rate during the step).

In normal MVN neurons, spike generation is highly linear over a wide range of input amplitudes and has highly stereotyped dynamics including little decrease in firing rate current step. Both application of a 11 Hz external canal blocker calcium (Ca, 100 nM) had a pronounced effect on spike generation in MVN neurons. First, the relationship between current and firing rate became non-linear in the presence of Ca, a given amount of current produced smaller changes in firing rate as the current amplitude increased. Second, spike generation gain increased, relative to control, at low current amplitudes. Finally, Ca altered spike generation kinetics; firing rate decreased markedly during current steps. The small conductance calcium-activated potassium (SK) channel cannot be solely responsible for these effects: blocking the SK channel with apamin (100-200 nM) produced a smaller effect on spike generation gain with no effects on linearity or dynamics. These results demonstrate that calcium influx into MVN neurons modulates spike generation gain, dynamics, and linearity and suggest that regulation of calcium-dependent processes could underlie adaptive changes in the gain and dynamics of the vestibulo-cerebellar reflex.

752.13 METABOTROPIC GLUTAMATE RECEPTORS IN THE MEDIAL VESTIBULAR NUCLEUS IN VITRO. P.P. Smith*, C.S. Darlington. Dept. of Psychology, Univ. Otago, Dunedin, New Zealand.

The aim of the present study was to examine the response of guinea pig medial vestibular nucleus (MVN) neurones in brainstem slices to the selective metabotropic receptor agonist, 1S,3R-amino-cyclopentyl-1,3-dicarboxylate (ACPD). Extracellular recordings were made from single MVN neurones using standard in vitro techniques. 60% (12/20) of MVN neurones responded to 1S,3R-ACPD at a concentration of 10 M, compared to 45% (8/20) and 35% (7/20) of neurones at concentrations of 10-2 and 10-3 M, respectively. These increases and decreases in firing rate were observed in different neurones; in general, the magnitude of the response was greater, and the duration of the responses was long. Recordings from a single slice which contained only the MVN confirmed that these responses were produced by the action of the metabotropic agonist within the MVN itself.

752.14 EFFECT OF VESTIBULOCEREBELLUM AND INTERACTION OF NECK INPUT WITH VESTIBULAR INPUT TO THE CAUDAL VESTIBULAR NUCLEI. D. B. Thompson, H. Haggard, R. H. Schor and Y. V. Wilson, The Rockefeller University, New York, NY, 10021.

Previously, we studied the vertical vestibular input to neurons in the caudal half of the descending and medial vestibular nuclei (CVN) in decerebrate cats with the caudomedial cerebellum removed. In the present study we investigated whether CVN neurons respond differently to vestibular stimuli when the caudal cerebellar vermis is intact. Little influence was observed. As in the partially decerebellated preparation, neurons classified as receiving input primarily from otoliths displayed, on average, a modest gain increase (stereotyped per se) and a dramatic increase that led position by 33-39° over the range of 0.05-1 Hz whereas central canal neurons displayed a gain slope of 19 per decade and phases that led velocity by more than 30°. In both preparations, the positions and orientations of canal neurons were clustered near the canal planes and those of otolith neurons tended to be in the roll quadrants. The CVN direct projections from the otoliths differ from those of the vertical system, and we further examined the responses of neurons in this region to natural neck rotation and to combined vestibular-neck stimulation. Only 30% of neurons responded to neck rotation in the range of 0.5 Hz. We investigated neck-vestibular interaction in 17 neurons that showed neck and vestibular responses with phase differences <45°. Only 7/17 neurons had “antagonistic” neck and vestibular vector orientations (i.e. differing by >10°) and 3/17 neurons had “synergistic” vectors (i.e. differing by <60°). In contrast, >50% of neurons in other rostral areas of the vestibular nuclei receive neck input, which is almost always antagonistic. Supported by NIH (DC 01217 and NS 24930) and by HFSPO.

752.15 DYNAMIC RESPONSES OF VESTIBULAR NUCLEUS NEURONS AND PREPOSITUS NEURONS IN THE ALERT GERBIL. G.D. Kaufman* and A.A. Perachio. Departments of Otolaryngology, Physiology & Biophysics and Anatomy & Neurosciences, Univ. TX Med. Br., Galveston, TX 77555-1063.

The responses of neurons in the nucleus prepositus hypoglossi (PH) were recorded in alert gerbils during horizontal angular or linear head acceleration in the dark. Angular acceleration, ranging from 0.05 to 2.0 Hz, 30°/sec to 120°/sec., was applied in the plane of the horizontal semicircular canals. At frequencies below 0.5 Hz, phasic bursting occurred during the otherwise small phasic portion of the cycle in some PH neurons. From 0.2-2.0 Hz gain increased in the horizontal plane. Responses to the horizontal plane were recorded from single PH neurons using standard in vitro techniques. 60% (12/20) of PH neurons responded to 1S,3R-ACPD at a concentration of 10 M, compared to 45% (8/20) and 35% (7/20) of neurones at concentrations of 10-2 and 10-3 M, respectively. These increases and decreases in firing rate were observed in different neurones; in general, the magnitude of the response was greater, and the duration of the responses was long. Recordings from a single slice which contained only the MVN confirmed that these responses were produced by the action of the metabotropic agonist within the MVN itself.

752.16 VESTIBULAR NUCLEI NEURON RESPONSE DYNAMICS PRODUCED BY COMMISSURAL CONVERGENCE IN PIGEONS. J.D. Dickson*, Dept. of Surgery (Otolaryngology), Anatomy, and Physiology, University of Mississippi Medical Center, Jackson MS 39216.

How do vestibular nucleus neurons synthesize the information from the paired semicircular canals into a unified output signal? To answer this question, the dynamic properties of vestibular nucleus neurons in pigeons were examined by individual and paired stimulation of the horizontal semicircular canals using both mechanical micropusher and neuronal stimulation. Extracellular single fiber responses from horizontal canal-related neurons were obtained in awake decerebrate birds that were paralyzed (Pavulon) and ventilated (250 ml/min, O2/CO2). Mechanical micropusher stimulation consisted of 10 Hz, 2.5 μm of the exposed ipsilateral horizontal membranous duct was delivered first, followed by identical contralateral canal stimulation. Bilateral mechanical stimulation was next delivered, with left and right canal stimulation 180° phase displaced. Rotational stimulation (0.1 - 4 Hz, 20 deg/sec) was finally delivered as a comparison. All Type I neurons responded to ipsilateral canal stimulation with increasing gain and phase advance to ipsilateral canal stimulus. Contralateral canal stimulation produced responses with much smaller gains and phases that were generally phase displaced by 180°. Two different response patterns were observed with bilateral canal stimulation with gains from ipsilateral and contralateral canal responses that were additive. Phase differences in the ipsilateral and contralateral canal high frequency stimulation were correlated with both gain and phase, with phase differences greater than 180° resulted in subtractive gains. These phase differences suggest that spatio-temporal convergence between inputs from the two complementary labyrinths exist for vestibular nuclei responses and be utilized to produce the total output signal that is projected to the target cell.
572.17 RESPONSES OF SECONDARY VESTIBULAR NEURONS TO MECHANICAL VESTIBULAR STIMULATION IN THE TOADFISH OPSINUS TAU. A. F. Menjinger* and S. M. Hightstein, Dept. of Otolaryngology, Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The responses of the toadfish horizontal canal afferents have been classified into three rough groups (high- and low-gain velocity sensitive, and high-gain velocity sensitive). The toadfish and the turtle vestibular nucleus (VN) during rotation of an in vitro turtle brainstem in which the temporal bones remained attached. The lateral canals were roughly aligned with the horizontal plane, and the horizontal axis of rotation was centered between the lateral canals. This preparation responded to vestibular stimulation for up to several days. In some preparations, horizontal canal sensitivity was confirmed by plugging the vertical canals or stimulation about the interaural axis.

Single VN units were isolated when they responded to horizontal sinusoidal stimulation and then quantified for different stimuli. Spike histograms were averaged for many stimulus cycles and fit to a rectified sine function. VN units were then categorized as encoding stimulus velocity or acceleration in the ipsi- or contra-rotatory direction.

Common features of these neurons were their low levels of spontaneous activity and their rectified sinusoidal responses to sinusoidal rotation. Each cell had a characteristic phase (re table velocity) that was invariant below 1 Hz stimulation. Response phase varied substantially between cells, with most phase values between 45° lag to a 90° lead. The response gain (re table velocity at 1 Hz) was between 0.1 and 25, indicating substantial heterogeneity of these cells' responses. These response features do not correspond to that of turtle vestibular afferents and VN cells in other species. (Supported by EY 05978 to MA)


The vestibular input induced by rotation of the animal in a given direction produces an appropriate postural adjustment. Whether the pattern of the vestibulospinal (VS) reflex recorded from the forelimb extensor triceps brachii (TB) could be modified by a relative body position during the animal's movement, as expected in order to preserve body stability, in decerebrate cats, the multiteminal VSG neuron of the TB was recorded during wobble of the whole animal at 0.15 Hz, 10°. This stimulus allowed the cat to maintain head stability, corresponding to the direction of head displacement leading to the maximal EMG response. When the body was kept still, only with respect to the head, the response vector of the VN was always oriented close to the transverse axis of the body. A phase shift of 10° of body-to-head displacement around a vertical axis passing through the atlanto-occipital joint, the response vector of the VN shifted in the same direction of body rotation, thus remaining approximately perpendicular to the body axis. The vestibular response vector was consistently reduced by inactivation of the cerebellar anterior vermis following microinjection into lobule V (culmen) of the GABA-A agonist muscimol (0.5 μl at 8 μg/ml saline). These findings indicate that l) the sensory input of the cerebellar origin is able to modify the pattern of the VS reflex which appears to be organized in a body-centered reference frame; 2) the cerebellar vermis is required for the proper execution of this sensorimotor transformation.


Axonal trajectories and locations of the cell bodies of the utricular (UT) and saccular (SAC) activated vestibulospinal neurons (VSNs) were studied in degerebrated or anesthetized cats. Bipolar stimulating electrodes were placed on either the UT or the SAC nerve with the other vestibular nerve branches transected. Bipolar stimulating electrodes were positioned in the lateral vestibulospinal tracts (LVS) bilaterally and the medial vestibulospinal tracts (MVS) at the C1-2 level. Lateral tracts were bilaterally inserted into the bundle of Voluntary fibers at the C2-4, C4-7, L3, and into the third nucleus. The majority of UT-activated VSNs were studied in the rostral part of the descending vestibular nucleus, while the SAC-activated VSNs were studied in the nucleus intercalatus and the anterior vestibular nucleus. Almost none of UT- and SAC-activated VSNs were activated from the third nucleus. The numbers of UT-VSNs (n=46) and SAC-VSNS (n=46) activated from each spinal segment were as follows:

<table>
<thead>
<tr>
<th>Spinal segments</th>
<th>C1</th>
<th>C2</th>
<th>C4-7, L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-LVS utricular</td>
<td>32</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>i-LVS saccular</td>
<td>14</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>MVST utricular</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>MVST saccular</td>
<td>29</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>c-LVS utricular</td>
<td>8</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>c-LVS saccular</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>


Department of Psychology, University of Alberta, Edmonton, Alberta, Canada T6G 2H9.

The generation of compensatory eye movements in response to rotational head movements involves the transformation of visual-oculomotor and vestibular signals into commands controlling the appropriate eye muscles. The oculomotor information is analysed by the Accessory Optic System (AOS). It has been demonstrated in rabbits and pigeons that the three systems (AOS, vestibular, eye muscle) share a common three-dimensional reference frame. This spatial reference frame in the oculomotor system is organized with respect to the eye muscles rather than the vestibular canals. Measurements of the plane of the horizontal eye movements obtained using standard extraocular techniques were recorded from two structures in the pigeon AOS: 1) the posterior nuclear layer lateral mesencephalon (LM), and 2) the nucleus of the basal optic root (nBOR). The LM and nBOR common response properties are consistent with nBOR moving forward and backward in the contralateral hemifield, respectively. Thus, activity in the LM and nBOR result in contraction of the contralateral medial rectus (MR) and lateral rectus (LR), respectively. We also recorded from Purkinje cells in the thalamus of the vestibulocerebellum (VSC). Neurons in the VSC have binocular receptive fields (RFs). The ipsilateral and contralateral RFs respond to OLS moving forward and backward, respectively. Thus, activity in the VSC neurons results in contraction of the ipsilateral MR and contralateral LR, respectively. A close correspondence was found between the plane of the MR, the direction preferences of LM neurons, and VSC neurons in response to stimulation of the ipsilateral hemifield. In all cases the vectors were aligned approximately with the horizontal. A close correspondence was also found between the plane of the LR, the direction preferences of nBOR neurons, and VSC neurons in response to stimulation of the contralateral hemifield. In all cases the vectors were tilted downward from the horizontal by about 30°. In sum, there is a close correspondence between the planes of the horizontal eye muscles and the direction preferences of AOS and VSC neurons providing oculomotor input to those muscles.
753.3
EFFECTS OF MESENCEPHALIC RETICULAR FORMATION (MRF) ELECTRICAL MICROSTIMULATION UPON EYE MOVEMENTS IN PRIMATE MONKEYS.* D.Guitton, J. Jamar, J. S. Timmerman, Newington VAMC, 555 Willard Ave, Newington, CT 06111.

While electrical microstimulation (MCS) is known to generate convergent horizontal eye movements (HEMs) in two fixed models. The monkeys sat in a dark room and fixed a small spot of light which either remained lit or was extinguished just prior to MCS. Sixty sites were across the rostral-caudal extent of the MRF were explored. At 23 sites IP was varied across a 3 or 5 point grid which contained the ocular range of 10° steps. Saccades from 0.5° to 25° amplitude were elicited. Direction of elicited EMs varied from primary horizontal to vertical in the more rostral regions. At 60% of the sites at which IP was varied, there was a distinct correlation of IP with elicited saccade amplitude (F > 0.5) such that shifts of IP toward the direction of the movement (as defined from primary position) were associated with smaller elicited amplitudes. A commensurate change in saccade direction accompanied the reduction in saccade amplitude. The large changes in EMs following MCS suggest that the MRF, distinct from the superior colliculus, may be organized in a craniotopic coordinate system.

Supported by NIH Research Grant EY 09481 and a RAG grant from the Office of Medical Research, Dept. of Veterans Affairs.

753.5
THE NITRIC OXIDE :-cGMP PATHWAY CONTRIBUTES TO THE CONTROL OF HORIZONTAL EYE MOVEMENTS PREPULSITUS HYPOGLOSSAL MOTONEURONS. M. Escudero*, D. Moreno-Lopez, and C. Estrada*. Laboratory of Neuroscience, Faculty of Biology, University of Sevilla, and Department of Physiology, Faculty of Medicine, UAM, Madrid, Spain.

Nitric oxide (NO) is a diffusible gas synthesized by some neurons in the central nervous system, with an intercellular communication function, which is mediated in part by cyclic guanosine monophosphate (cGMP) in target cells. The possible role of nitric oxide as a modulator in the cat oculomotor system was investigated based on the large number of neurons containing NO synthase (NOS) in the posterior hypothalamic nucleus (PH). Alert cats prepared for chronic recording of eye movements by means of the magnetic search-coil technique were used. Local injections of the NOS inhibitor L-NAME (40-60 nmol) and L-N-monomethylarginine (L-NMMA; 40-60 nmol) in the anterior third of the PH produced conjugated nystagmic eye movements with slow phases directed to the contralateral side, and spontaneous eye movement alteration was dose-dependent, appeared within 1-2 minutes after injection, lasted for 30-60 minutes and was more evident in complete darkness. The effect of NOS inhibitors was stereotyped, which is specific for the posterior hypothalamic nucleus (PH). Eye movements were affected, and was abolished by simultaneous administration of the NOS substrate, L-arginine. Intravenous IP of the NO donor sodium nitroprusside (SNP) or the permissive analog of cGMP 8-bromo-cGMP in the PH produced an effect opposite to that of NOS inhibitors, this is, conjugated nystagmic eye movements with slow phases directed to the ipsilateral side. These results indicate that NO produced by PH neurons modulates via cGMP the generation of the motor signal that control horizontal eye movements.

This work was supported by grant 94/0388 from FIS, Spain.

753.7
ANALYSIS OF PRIMATE INHIBITORY BURST NEURON SPIKE TRAIN DYNAMICS. GAZE VERSUS EYE BASED MODELS OF EYE-HEAD GAZE SHIFTS. E.E.Cutler* and D.Guston. Aerospace Medical Research Unit & Montreal Neurological Inst. McGill University, Montreal, Canada.

We used metric analysis and systems identification techniques to relate IB discharge to the dynamics of gaze, eye, and head during head-free gaze shifts. Burst versus gaze durations were similar head-fixed and head-free, and the number of spikes was better correlated to head-free gaze, than eye delay. Latencies calculated using a simple head-fixed model were significantly shorter than those derived from the onset of the first spike in head-fixed and head-free conditions. Regardless of an IB's onset delay, simple downstream models were better than upstream models (non-linear function of motor error) at predicting IB discharges. The most important parameter to head-free downstream models was a velocity gain coefficient and a bias term. For all models with a bias term, gaze and eye velocity were important for generating IB activity, however the bias term estimated for eye based models was significantly higher than that for gaze based or head-fixed models. Head velocity based models provided worse fits than gaze or eye velocity based models. Acceleration and higher order non-linear velocity terms slightly improved the variance accounted for in models (10%), whereas, of course, the analogue of the IB firing rate greatly improved model fits, when initial conditions (ICs) were estimated as parameters (15%). However, these models yield bias terms that were unrealistically negative. Models with only a gain and bias term, in which the bias term was estimated separately for each gaze shift were compared to those with a palin term with estimated ICs. Estimated biases were inversely correlated with the peak gaze velocity and/or amplitude of the head-free eye movement component for only 18% of IBs (eye base models). In contrast, these values were well correlated with peak velocity and/or amplitude of the head-free eye movement component for only 18% of IBs (eye base models). The model did not predict the bias magnitude of model fits: (1) the bias enables a distinction between head-fixed and head-free fits, and suggests that gaze velocity is more relevant than eye velocity in describing IB discharges during gaze shifts 2) the bias is strongly related to the metrics of saccades head-fixed and the metrics of gaze, not eye, head-free.

753.8
NEURAL NET SIMULATION OF EYE POSITION COMMAND TO MEDIAL RECTUS MOTONEURONS FROM ADUCBUCS INTERNUCLEAR NEURONS. Paul Dept*, Dept. of Psychology, University of Sheffield, Sheffield S10 2TP, England.

Ocular mononeuron firing rate is linearly related to eye position (in the relevant direction) with slope K, above recruitment threshold. Within the population of ocular motoneurons K increases as T increases. It is not known how these relations are derived from the combination of input from several intrinsic motoneuron properties.

Possible derivations may be investigated by simulating the input signal to medial rectus motoneurons (MR-MN) from intracranial neurons of the abducens nucleus (AIN). AINs were represented as input neurons in a two layer neural-net, each with weights representing the afferent connections of an MR-MN. The output node bias term acted as the intrinsic MR-MN threshold. Inputs to the net were conjunctive eye-position commands, used to generate realistic firing patterns in the AINs (Fuchs et al., J.Neurophysiol. 50:1874, 1988; Lamml et al., J.Neurophysiol. 62:70, 1989). The output of the net was compared with actual MR-MN firing patterns for that eye position (Garvin & May, J.Neurophysiol. 67:64, 1992). Weights were adjusted as a result of the comparison using gradient descent.

The simulations showed: (1) MR-MN firing rates were accurately reproduced by AIN populations in which R was unrelated to recruitment threshold T, and in which maximum firing occurred in the positive quadrant of those in the MR-MN population. (2) Accuracy could still be achieved when the bias term, representing MR-MN intrinsic threshold, was held constant. (3) Weights between AINs and MR-MN showed that each MR-MN typically received functional connections from a cluster of AINs with similar T. It is therefore possible that appropriate patterning of input connections can determine MR-MN firing rates in relation to conjunctive fixation commands, without a major contribution from variation in intrinsic motoneuron properties.

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753.4

Previous single unit units in RM/MRF III have emphasized that the pulse of activity observed in oculomotor neurons (OMNs) is stereotypic and the frequency of the instantaneous discharge during the OMN pulse controls eye velocity. The discharge dynamics of 43 neurons within III and 31 horizontal, saccade related MRF neurons were recorded in 2 awake, head-fixed monkeys performing visually guided saccades. Neuronal discharge frequencies were expressed as a spike density function (SD) and individually matched to the temporal dynamics of individual saccades. Motor error (ME) and velocity error (VE) showed a commensurate decline of SD with declining motor error. Phase plane plots of SD versus VE eye velocity generated an open loop indicating a different level of activation during the accelerating and decelerating phases of the saccade. Velocity (VE) neurons (MRF=16 and III=11) had a "ski-jump" decline of SD with declining ME. These cells showed a closed loop response for the SD versus VE phase plane. The remainder of the neurons demonstrated a mixture of these two responses. In light of similar responses of saccade related cells in the superior colliculus, these results suggest that both VE and ME are controlled variables at multiple levels of the primate oculomotor system. Supported by NIH Research Grant EY 09481 and a RAG grant from the Office of Medical Research, Dept. of Veterans Affairs.
753.9

The relative importance of genetic and epigenetic factors in developmental regulation of the unique extraocular muscle (EOM) phenotype is unclear. The \textit{Wnt-1} allele is required for the development of the muscles, including the oculomotor and trochlear nuclei. \textit{Wnt-1} mutant mice, by homologous recombination (McMahan and Bradley, Cell 62:1075, '96), may then represent a second genetic model in which to examine the role of neuromuscular interactions in developing EOM. Prior studies have shown that the EOM primordia are innervated prior to migration away from their somatotopic origins. However, innervation may not be a critical factor in guidance of migration since \textit{Wnt-1} mutants showed normal spatial orientation of the six EOMs within the orbit at E14.5-16.5 in spite of the absence of oculomotor and trochlear motor neurons. Lateral rectus muscles contained axonal and primitive neuromuscular contacts from the intact abducens motor neurons. However, some of the muscles normally innervated by oculomotor and trochlear nuclei received motor innervation from an alternative source, presumably via collateral sprouting of abducens motor neurons. Muscles receiving either normal or aberrant innervation exhibited myogenic staging comparable to control littermates. By contrast, muscles lacking evidence of innervation contained atrophic and degenerating myocytes. The pattern of pathology in aural muscles suggests that innervation from oculomotor nerves plays a role in both primary and secondary myogenesis in this muscle system. Taken together, this model may allow characterization of the mechanisms responsible for the tissue-specific properties of the EOM alotype. The pattern of developmental aberrant innervation of EOMs seen in this mouse also may provide insight into the etiology of Duane retraction syndrome in humans. Supported by NIH EY09384 and Research to Prevent Blindness.

753.10
THE PRETECTAL NUCLEUS OF THE OPTIC TRACT (NOT) SUBSERVES LATENT NYSTAGMUS IN VISUALLY DEPRIVED MONKEYS. M.J. Mustari1,2, J.F. Fuchs, J.A. Tusa, A. Barlow, and C. Livingston. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX; 1Regional Primate Research Center, University of Washington, Seattle, WA, 2University of Miami Ear Institute, Miami, FL.

Monkeys (Macaca mulatta) binocularly deprived (BD), by lid suture, early in life, develop a permanent horizontal nystagmus, resembling latent nystagmus (LN). These animals also lack a naso-temporal opokinetic reflex (OKN). To discover the locus for LN, we recorded from and reversibly inactivated the NOT, which is essential for horizontal opokinetic eye movements. We recorded NOT unit activity during spontaneous nystagmus and fixation, and evaluated visual receptive fields in two trained monkeys (> 2 yr old), which had been BD for the first 25 or 55% of life. NOT units in BD animals were either driven exclusively (55 day BD) or were dominated (62%; N=32) by the contralateral eye (25 day BD). This is in contrast to normal monkeys where all NOT units are binocularly driven. In addition, most NOT units changed their spontaneous firing rates as a function of ambient light levels. Such light intensity dependent firing, together with contralateral ocular dominance, could be the source of an unbalanced drive to caudal brainstem structures, resulting in LN.

To test this suggestion, we placed injections of the GABAa agonist, muscimol (0.1-5% in the NOT. Prior to muscimol injection, LN was initiated 145ms (515) after closing a shutter in front of the right eye, with rightward slow phase eye velocity reaching 40°s in a few seconds. Following unilateral muscimol blockade of the NOT, ipsiversive (slow phase) LN due to inactivating the ipsilateral eye was completely abolished. Similarly, bilateral NOT blockade abolished LN in either direction. Horizontal opokinetic eye movements were virtually abolished following bilateral injections, however, the monkey’s ability to fixate or make saccades was preserved.

The results of our studies indicate that the NOT is critical for the production of LN in animals subjected to brief periods of visual deprivation early in life. 1Tusa et al., Invest. Ophth. Vis. Sci. 32: 134-141, 1991. 2Mustari and Fuchs, J. Neurophysiol. 64:77-90, 1990. Supported by: EY0699, EY09289, RR 0166, EY07074.

753.11

Judged by the number of neurons that can be visualized with retrograde transport of axonal tracers, the dorsomedial pontine reticular formation is one of the major sources of mossy fibers to the flocculus and ventral parafasciculus. Several studies in rat, cat, rabbit and monkey demonstrated in and around the mf a population of so-called paramedian tract neurons (PMT) with a size similar to that of the population of "secondary" vestibulo-cerebellar mossy fiber neurons. In the macaque (M. fascicularis), approximately 40 percent of the PMT’s is located in two clusters, one ± 2 mm caudal to the facial genu, the other directly rostral to the abducens nucleus. The majority of PMT neurons are dispersed between the bundles of the mf.

Injections with WGA-HRP in the medial (MV) and superior (SV) vestibular nuclei resulted in wide-spread labeling of axon terminals in the entire region containing PMT neurons. Since the ascending connections of the MV and SV are basically related to horizontal and vertical eye movements (EM), respectively, the results suggest an intermingling of horizontal and vertical EM related PMT neurons.

753.12

We have recently shown that many vertical eye position-related neurons in and around the interstitial nucleus of Cajal (INC) project to and arborize in the ipsilateral superior vestibular nucleus. In the present study, we investigated the alert cat possible projections of vertical eye position-related INC neurons to the abducens nucleus. In two animals, 37 of 113 neurons were activated antidromically from the trochlear nucleus or nearby MLF. Most activated neurons (33/37) had downward on-directions. We performed tracking through the trochlear nucleus to obtain the depth profile of thresholds for antidromic activation. Multiple low threshold peaks and variation in latency were observed in some neurons with upward on-directions, suggesting direct projection of downward-on INC neurons to trochlear motoneurons. Since interstitial vestibular neurons also had downward on-directions, there is a possibility that some INC neurons project to both the trochlear and vestibular nuclei. We then made an attempt to decide the synaptic nature of interstitial vestibular neurons by spike triggered average of field potentials in the superior vestibular nucleus. In three eye position-related neurons with downward on-directions, unitary synaptic field potentials with a negative polarity were detected at monosynaptic latencies, suggesting excitatory actions. The results suggest that vertical eye position-related interstitial vestibular neurons exert excitatory synaptic actions upon both vestibular nucleus neurons and trochlear motoneurons.

753.13
EFFERENT PROJECTIONS OF THE PRIMATE INTERSTITIAL NUCLEUS OF CAJAL. A.K. Hochschwab*, C. Krugler, G. Halban, S.M. Hirschsteiner and T. Kohikoyayama, Laboratory of Neurophysiology, Dept. of Basic Sciences, P.O. Box 1393, Faculty of Medicine, University of Crete, Iraklion 71110, Heraklion, Greece; Eye and Ear Institute, Pittsburgh University, Pennsylvania, U.S.A., and Department of Otalaryngology, Washington University School of Medicine in St. Louis, Missouri, U.S.A.

The efferent projections of the mesencephalic Nucleus Interstitialis of Cajal (NIC) were studied in the squirrel monkey following bulk injections of ibotenic acid and PHA-L near functionally identified oculomotor related burst-tonic neurons of the NIC. Dense terminal fields were encountered: a) contralaterally, in the NIC, the oculomotor nucleus and the trochlear nucleus, and b) ipsilaterally, in the fields of Forel, the rostral interstitial nucleus of the medial longitudinal fasciculus, the oculomotor and trochlear nuclei, the gigantoocular recticular formation, as well as the ventromedial and commissural nuclei of the face, the cervical segments of the spinal cord. Moderate or weak terminal fields were observed: a) bilaterally, in the mediodorsal, central median, central medial and parafascicular thalamic nuclei, and b) ipsilaterally, in the zona incerta, the mesencephalic recticular formation, the pedunculopontine nucleus, the nuclei reticularis pontis oralis and caudalis, the superior, medial, and lateral vestibular nuclei, as well as the n. prepositus hypoglossi, the abducens and the hypoglossal nucleus, the mammocerebellar recticular formation, the inferior olives as well as the pontine and medullary raphes.

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The topographical location of brainstem and deep cerebellar neurons projecting to the eyelid microzone of the simplex lobule was studied in the cat. Unilateral cerebral anesthesia, horseradish peroxidase (Sigma type VI, 1-5-µL of a 30% saline solution) was injected in six adult cats in the area of the simplex lobule activated by the electrical stimulation of the supraorbital branch of the trigeminal nerve. After 24-48 h of survival, animals were perfused and their brains processed according to the tetramethylbenzidine procedure. Deep cerebellar nuclei neurons projecting to the eyelid microzone were located in the dorsomedial aspect of the most rostral part of the posterior interpositus nucleus. Contralateral labeled brainstem neurons were located in the spinal trigeminal and prepositus hypoglossi nuclei, in the rostral medial accessory inferior olive, and in the lateral and dorsal subdivisions of the pontine gray. Ipsilateral projections arrived from the lateral reticular, the external cuneate nuclei, and a discrete zone of the medial pontine gray. Bilaterally labeled neurons were placed in the lateral tegmental area, the paramedian reticular nucleus, and the reticulotegmental pontis nucleus. This complex pattern of afferents suggests that the simplex lobule plays a key role controlling the different types of lid movement.

ACTIVITY OF BRAINSTEM NEURONS DURING BLINK-SACCADE INTERACTIONS. L. E. Mayo* and D. W. Morrisey, Dept. of Physiological Optics and Vision Science, Brain Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Recently, we have shown that pontine omnipause neurons (OPNs) in the monkey pause for blinks as well as for saccades (Soc. Neurosci., p. 1404, 1993). Blink-related pauses last 140 ms and are easily elicited by air puffs directed at the face. Current models of saccade generation suggest that neuronal activity builds up in other saccade-related neurons prior to each saccade, and that turning off OPNs prematurely during this build-up period will result in slow saccades. We tested this hypothesis in three rhesus monkeys by timing the delivery of an air puff to produce a blink before, during, or after a saccade to a visual target. The occurrence of a blink just before a saccade resulted in a slowed saccade, often with a complex velocity profile. Blinks which occurred >70 ms prior to the expected saccade abolished the blink of the saccade. Horizontal short-latency burst (SLB) neurons were recorded during blink-saccade interactions in two monkeys. SLB activity was reduced for those saccades that were slowed by blinks. The slowing of saccades by blinks is not due simply to co-contraction of extracranial muscles or other mechanical effects. Surprisingly, the activity of saccade-related burst neurons in the superior colliculus was also reduced for blink-slowed saccades. (Supported by NIH grants EYO3463 and EYO3039 and by the McKnight Endowment Fund for Neuroscience.)


Unilateral destruction of midbrain dopamine neurons dramatically increases the excitability of the blink reflex by reducing tonic inhibition of the trigeminal nucleus. Increases in blink reflex excitability also occur as a compensatory response to weakening of the orbicularis oculi muscle (OO). We investigated the relationship between these two mechanisms of blink reflex plasticity. Rats were implanted with electrodes to stimulate the suprorbital branch of the trigeminal nerve (SO) bilaterally and record the activity of OOemg (EJS). Secondary excitability was measured by presenting pairs of identical SO stimuli with interstimulus intervals of 50-300 ms and comparing the magnitude of the OOemg evoked by the second stimulus to that evoked by the first. Interstimulus intervals of 50 ms and auditory-evoked blinks were also assessed. We tested blink reflex excitability in two groups of alert rats. In both groups, we removed a 2 mm section of the facial nerve innervating the OO. The second group received a small, unilateral 6-OHDA lesion (<20% cell loss) of midbrain dopamine neurons 20 days before the nerve transaction. Both 6-OHDA lesions and facial nerve lesions alone produced modest increases in blink reflex excitability. The combination of the 6-OHDA and facial nerve lesions, however, elicited a large increase in blink reflex excitability accompanied by lid closure spasm such as occur with blepharospasm. Interacting auditory and SO blinks revealed that the facial nerve lesion exerted its greatest effect on trigeminal evoked blink. Thus, the data suggest that dopamine neuron loss potentiates the normal compensatory increase in trigeminal nucleus excitability induced by weakening of the OO muscle.

SUPPORT: By EYO7391 (CE) and IT32NS07371 (EJS).


Neurologic disorders that affect blinking (e.g., blepharospasm, Parkinson disease) often manifest at ≥50 years of age. Eyelid kineismics provide insight into the altered blink reflex excitability that may accompany disease. To evaluate the normal aging pattern, we used a modified scleral search coil technique to analyze blinks in normal human subjects for each decade between 40-90 years (n=5 decade). Mean amplitude and peak velocity decreased across this interval (amp: 38.8 ± 4.4 to 26.0 ± 2.8, p<0.05; vel: 1200/sec ± 68 ± 182/sec ± 131, p<0.05) and up (amp: 36.0 ± 5.3 to 24.2 ± 3.4, p<0.05; vel: 587/sec ± 43 to 439/sec ± 72, n.s.) phases of spontaneous blinks. Much of the decrease could be attributed to reduced palpebral fissure width (12.4 ± 2.0 mm, p<0.05). Despite down phase amplitude reduction of ≥30%, down phase duration remained constant. Blink down phase main phase (amplitude-peak velocity) slope is an indicator of reflex excitability. There was no change in main sequence slope across the age range tested, suggesting that reflex excitability was unchanged. Blink conjugacy also showed no change, with consistently high interocular correlations for amplitude/peak velocity across the intervals studied. Blink rate was unchanged across the age range tested. Together, these studies evaluated the hypothesis that blink excitability changes as a function of age, with the hypersensitivity that is seen in blepharospasm and Parkinsonism representing extremes of normal aging. The peripheral eyelid and/or muscle changes leading to age-related ptosis were not accompanied by altered reflex excitability. These data then suggest that disease-related alterations in blink behavior and, by correlation, in the blink neural control systems, are not simply an exaggeration of normal aging changes. Supported by NIH EY07160 and the Basic Essential Blepharospasm Research Foundation, and Research to Prevent Blindness.

EFFECT OF BILATERAL LID REPOSITIONING ON BLINK-SACCADE COORDINATION IN RATS. M.J. de Lourdes, J. Smith, A. Wair, Deps. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

As background for studies to examine the modification of the blink reflex by drugs or manipulation of central pathways, behavioral studies were conducted in seven alert, adult male cats. Under anesthesia, the animals received chronic stimulating electrodes over the supraorbital nerve and recording electrodes in orbicularis oculi muscle for electromyography (OOemg). With a stimulus duration of 0.1 msec and a stimulus magnitude of 1.5X threshold, measures of latency, duration, area, and maximum amplitude (MA) were obtained. The cat, like a human being, should have a 20 msec latency (R2) and a long latency (R2) component in the OOemg. Similar to humans, the mean (+ SE) latency for R1 in cat was 10.8 ± 2 msec and for R2, 37.0 ± 1 msec. For both R1 and R2, MA and area measures were significantly correlated. Only area and MA of the R1 and R2 showed significant change as a function of stimulus intensity. The pain stimulus paradigm in which the interstimulus interval (ISI) was varied from 100-1200 msec, ratios were constructed for the Ooemg area and MA by dividing the test response by the conditioning response. In this paradigm, a significant linear relationship was observed only between ISI and R2 MA. (Supported by the Dystonia Med. Res. Fdn.)
FUNCTION-SPECIFIC SUBDIVISIONS OF THE NUCLEUS OF EDINGER-
WESTPHAL AS REVEALED BY TRANSMITTER-TRANSPORT OF WGA.
of Psychology, University of Maryland, College Park, MD 20742.

In most areas of the central nervous system, the axons of retinal ganglion cells project to specific regions of the brain in a highly ordered, topographic manner. Two recent studies have demonstrated the stereotypical localization of retinal ganglion cell axons in the superior colliculus of the primate. One study used retrograde axonal tracing to demonstrate that the retinal ganglion cell axons project to specific regions of the superior colliculus in a topographic manner. The other study used anterograde axonal tracing to demonstrate that the retinal ganglion cell axons project to specific regions of the superior colliculus in a topographic manner. The combination of these two studies provides a comprehensive understanding of the topographic organization of retinal ganglion cell projections to the superior colliculus.

LATENCY AND DYNAMICS OF PUPILLOCONSTRUCTION DETERMINED BY MICROSTIMULATION OF THE EDINGER-

We have been studying the neural control of the pupil in alert rhesus monkeys. To better characterize the latency and dynamics of the pupil, we have used the pupilloconstriction test which results from electrical microstimulation of the Edinger-Westphal nucleus (EW) or the oculomotor nerves (OMN). In rhesus monkeys fixated a dim laser spot on a tangent screen while the pupil was measured under infrared lighting using ISCAN RK-406 pupillometry systems. A microelectrode was lowered under physiological guidance either to the EW or to the OMN. Microstimulation was carried out over a wide range of parameters (100-1000 Hz; 10-100 µA; 3-10 ms). In response to brief stimulus trains, the pupils constricted with a latency of 50-100 ms. These responses were associated with higher stimulation currents. Peak pupilconstriction occurred approximately 250-500 ms after stimulation and showed an exponential return to baseline with a time constant of approximately 300-400 ms.

These characteristics indicate that the pupillary plant acts as a low pass filter and explain the sluggishness of pupillary responses. The first-order models of the pupillary plant that have been proposed do not adequately explain these results and a third-order model is required to provide a more complete description. (Supported by NIH EY05638 and P30 EY03039).

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754.13

DESCENDING PROJECTIONS FROM THE CORTICAL ACCOMMODATION AREA TO THE BRAINSTEM IN THE CAT.

Previous studies indicated that the lateral suprasylvian area (LS) is related to control of lens accommodation in the cat. It is known that the LS projects to the pretectum (PT) and the superior colliculus (SC). We previously indicated that both of the rostral SC and the PT are involved in the control of less accommodation. In this study, we investigated connections between the cortical accommodation area in the LS and the brainstem accommodation areas in the cat.

This study was conducted in 6 cats, weighing 2.5-3.5 kg. WGA-HRP was injected by glass micropipettes into the accommodation area of the SC or the PT where accommodative responses were elicited with the low intensity (< 20 µA) and studied retrogradely labeled cells in the LS. Secondly, accommodative responses to stimulation of the LS were compared before and after injection of muscimol (GABA agonist) into the rostral SC (RSC). Following WGA-HRP injections into the accommodation area in the rostral SC, labeled cells were found mainly in the lower part of the medial bank of the medial suprasylvian nucleus (MS5), which is comparable to the accommodation area of the LS. On the other hand, following injections into the accommodation area in the PT, labeled cells were found mainly in the upper part of the medial bank of the MS5, which is comparable to the pupillary constriction area. After injections of muscimol into the SC, accommodative responses evoked by stimulation of the cortex were almost abolished. These findings suggest that the accommodation area of the LS projects exclusively to the rostral SC.

754.14

ACCOMMODATION-RELATED AREAS IN THE BRAINSTEM OF THE CAT.

We previously indicated that the superficial–intermediate layers of the rostral superior colliculus is involved in the control of less accommodation in the cat (Sawa and Ohnaka, Vision Res., '94). This area is thought to project to the pretectum (PT) and the mesencephalic reticular formation (MRF), which may be related to the control of accommodation. In this study, we conducted systematic mapping with microstimulation of these areas monitoring accommodative responses by an infrared optometer in the cat. This study was conducted in 8 cats. Tungsten microelectrodes were introduced stereotaxically into the brainstem, and the midbrain area located rostral to the SC was stimulated sequentially. Accommodative responses were monitored with an infrared optometer (Nidek, AR-1100). At the end of each experiment, electrolytic lesion was made at the lowest threshold site. After the experiments, the animals were deeply anesthetized with pentobarbital sodium and were perfused transcardially. Locations of the electrolytic lesions and electrode tracks were identified. Low threshold areas for evoking accommodation were located at the postero-lateral portion of the PT, where was almost comparable to the nucleus optic tract (NOT), the olivary pretectal nucleus (OPN) and the posterior pretectal nucleus (PPN), the nucleus posterior commissure (NPC) and the medial portion of the MRF. The OPN and the MRF are also thought to be involved in the control of pupillo-constriction and vergence eye movements, respectively. Accommodation, pupillo-constriction and vergence are functionally linked with each other as the ocular near response.

754.15

INNERVATION OF THE SMOOTH MUSCLE OF THE EXTRACULAR RECTI PULLEYS IN HUMANS AND MONKEYS.

The recti extracocular muscles pass through connective tissue pulleys, located at the level of posterior Tenon's fascia, that stabilize muscle paths and control muscle pulling direction. Structures supporting these pulleys include substantial smooth muscle (SM) bundles, suggesting possible dynamic regulation of pulley function.

To investigate neural control of pulley SM, histo- and immunohistochemical studies were performed on human and monkey orbital tissues. Two weeks prior to paraformaldehyde injection, both superior cervical ganglia of a monkey were injected with the lectin anterograde tracer PHA-L. Immunoperoxidase staining with a monoclonal antibody against human SM alpha-actin was used to confirm pulley SM. Mono- and polyclonal antibodies were used to demonstrate immunoreactivity (IR) to tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), catechol-O-methyltransferase (COMT), myosin basic protein (MBP), PHA-L, neuronal nitric oxide synthase (NOS), and synaptophysin. The NADPH diaphorase reaction was also used as a marker for NOS, and the acetylcholinesterase (AChE) reaction for ACh.

Rich innervation was found in human and monkey pulley SM. Numerous axons terminating in motor endplates within SM bundles had IR to TH, DBH, and PHA-L but not COMT. Smaller axons and motor endplates were also demonstrated in SM using NADPH diaphorase and AChE, as well as NOS IR. The pargyline and at a lower extent the ganglion cells but not Edinger-Westphal nucleus had cells with NOS IR. Although large orbital nerves had MBP IR, none was found in pulley SM.

The smooth muscle suspension of monkey and human recti pulleys has a sympathetic projection employing norepinephrine via the superior cervical ganglion, a nicterosisogenic projection from the pargyline ganglion or other sources, as well as a cholinergic parasympathetic projection. These multiple projections suggest the existence of both excitatory and inhibitory control of recti pulley SM potentially subserving a dynamic role in regulation of ocular motility.

Supported by USPHS NEI EY-08313 and Research to Prevent Blindness.

755.1

SYNCHRONIZATION IN FINGER MOTOR UNITS DURING THE PRECISION GRIP IN MAN. E.J. Holder, G.C. Manley, M.A. Mozer and M.C. Hepp-Reymond*. Brain Research Institute, Univ. of Zurich, W129 Zurich, Switzerland.

Since the multi-muscular, multi-unit system of the hand is biomechanically overspecified, the question was raised whether short-term synchronization is a strategy to facilitate the control of motor units (MU) within and between muscles. Synchronization was assessed in the precision grip during production of isometric force on 3 consecutive levels (1.25 N). Up to 5 simultaneously recorded intramuscular myoelectric potentials were decomposed into constituent MU potentials yielding 93 MUs (1 to 4 MUs/muscle). The maximal firing rate in the force range investigated was 14 Hz. The force maximum of the MUs, the firing rate correlated with the force exerted. The MU spike trains were cross-correlated at each force level. In total, 75 of 166 intramuscular and 54 of 69 intramuscular MU pairs showed synchronization at any one force level. In pairs where both MUs were located in the extrinsic or intrinsic muscles, synchronization was stronger and more frequently observed. Furthermore, among MUs of intrinsic muscles, pairs between thumb and index fingers (33/66) were synchronized to a comparable degree as pairs between muscles moving only the center of the digit (12/23), thus indicating the presence of functional synergies. An important finding was that synchronization is unstable: it occurred on all 3 force levels only in one intramuscular (1D-1P) and in four intramuscular pairs (2DxAP, AP AP, AP FFL). We checked factors that might influence the probability of synchronization. Synchronized MUs had smaller differences between their firing rate (2 Hz) and lower firing rates than non-synchronized ones. Synchronization occurred preferentially just after recruitment of both MUs or of the MU with higher threshold.

In conclusion, stable synchronization of MUs does not seem to be a prerequisite for the control of finger muscles during force production in the precision grip, thus confirming previous findings at the global EMG level.

755.2

MODIFICATION OF PREHENSION KINEMATICS WHEN AVOIDING AN OBSTACLE M. Saling, J. Alberts, J. R. Bleedel and G. Steinbach*. Motor Control Lab., Arizona State University, Tempe AZ 85287, Barrow Neurological Institute, Phoenix, AZ.

Previously we had shown that, by altering the initial grip posture, the aperture reorganized without influencing the wrist trajectory. The aim of this study was to determine whether modification of the wrist trajectory affects the aperture adaptation. To examine this issue, either of two different plexiglass obstacles (20x1.5x9 cm or 20x1.5x 11 cm) were placed between the hand starting position and object. The subjects were asked to reach over the obstacle and grasp a dowel (9 x 2 cm). The results showed that obstacle avoidance significantly prolongs transport duration, deceleration time, time to peak velocity and peak deceleration. Also, the time to peak aperture and the time to the peak opening and closing velocities of grip were significantly longer compared to those of control. However, when expressed as a percentage of transport duration, no differences were found in any temporal parameter of the grip. Primarily these data show that the kinematics of both components were influenced by obstacle avoidance. The temporal aspects of the grip were adjusted to those of the transport and suggest some degree of co-dependence. These findings support a view that grip aperture adjustments are influenced by the temporal profile of the transport component.


Ramp loads that pull a gripped handle away from the hand cause the grip to smooth to track the load force after an initial grip force pulse triggered by the increasing load force. The tracking behavior in 9 subjects was analyzed to determine the control mechanisms governing pursuit-like tracking, and the saccharide-like pulse in grip force that often occur during tracking. We examined the possibility that subjects learn the smooth tracking response from the load shape on previous trials. Ramp loads of 4 Ns followed blocks of trials that began as a ramp load but which accelerated or decelerated after 0.75 s. The grip force during ramp loading was affected little or not at all by the conditioning loads. Also, the grip force closely tracked various load waveforms (ramps, slowly accelerating or decelerating loads). We conclude that the control process for the smooth-like behavior uses proprioceptive information from loading over a short time interval. Smooth tracking most likely results from a feedforward controller that continuously extrapolates the current waveform during short time intervals. It operates in addition to a mechanism that is sensitive to unexpected changes in load force rate, or otherwise decreasing grip-to-load force ratios. Loads that accelerate from an initial ramp often cause repetitive volatile grip responses lasting about 250 ms, beginning as soon as 80 ms after acceleration onset. We suggest that stable grasp on the object is maintained by one control mechanism that operates nearly continuously, and by one that responds to discrete mechanical events at the fingertip or finger.


Task requirements and object properties affect kinematics (Kunesch, Binkofski & Freund, 1989) and forces (Fearing, 1986; Westling & Johansson, 1990) in the execution of manipulation. We examined the transport, manipulation and manipulation that are able to perform a task requiring them in human-computer interaction.

Human adults grasped a dowel (155 g, force transducer) using pad opposition, and placed it on small or larger targets, forward or backward. They transported the dowel in three conditions: naturally, by "dragging" in transport during transport, and by "selecting" (selecting placement). 3D kineamtics of the hand and dowel (OPTOTRACK 3D motion analysis system, 200 Hz), grip and load forces were measured throughout the movement. A control motion, subject, was made from a position, load force, and motion phases had lengthened deceleration for smaller participants. A transport phase was not selected and "select" conditions; grip and load forces changed predictably with tasks, validating that subjects were performing as instructed; load forces during compliant motion peaked earlier for forward than backward transport. Results support anticipatory planning of parameters for using an opposition space (MacKenzie & Iberall, 1995) in the earliest phases of movement.

TIME-DEPENDENT EFFECTS OF ANTAGONIST MUSCLE VIBRATION ON THE FINGER FORCE. T.J. Fearing, K.L. Haninger, R. Heinengen, B. Endo-Henningsen, S.Koerts, A.M. Gordon. Dept. of Neurology and Neurophysiology, Univ. of Wisconsin, Madison, WI 53705; Dept. of Neurology, Univ. of Munster, Germany; Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455

We examined the contribution of agonist and antagonist muscle spindles to the perception and control of isometric finger force. Nine subjects were trained to produce a force level of 50 ± 25 g with the index finger. Following training, either the agonist (flexor digitorum superficialis) or antagonist (extensor digitorum) muscle was stimulated at 100 Hz for 5 sec prior to the onset of force. Agonist vibration resulted in an overshoot of the trained force level, likely due to autogenic reflex facilitation. Surprisingly, antagonist vibration resulted in a similar overshoot of the trained force level. To further examine the role of antagonist spindles in the control of isometric force, we then vibrated the antagonist at various time periods relative to the onset of the stimulated force. 4 sec prior to the onset of force, 2 sec before the onset of force, 4 sec after the onset of the dynamic force increase, and 4 sec after the onset of the dynamic force increase. We observed that a small vibration to the antagonist caused the largest overshoot of force if it occurred prior to the onset of the force. The overshoot diminished progressively as the vibration was initiated later, with no overshoot observed when the vibration started before the hold phase. We propose that sensory information from antagonist muscle spindles contributes to an internal model of the initial state of the effector prior to the onset of force, and that this information mainly is used for the feedforward control of isometric force.

DEVELOPMENTAL CHANGES IN PREHENSION FOR CHILDREN OF 2 TO 9 YEARS OLD. M. Piep and C. Dugas. 1- Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada, H3C 317, 2- Département des sciences de l’activité physique, Université du Québec à Trois-Rivières, Trois-Rivières, Canada, G9A 5H7

Anticipatory control is required to produce skilled manipulation of grasped objects. Adults program the entire movement with a single force rate pulse by anticipating the required grip and load force to lift the object (Johansson and Westling, 1988). Recently, Forsberg et al. (1991,1992) have demonstrated that children are not capable of doing so until the second year, and that this anticipatory mechanism continues to develop over several years. The goal of the study was to demonstrate that the emergence of an adult pattern of grip and load force was not achieved until the second year, and that this anticipatory mechanism continues to develop over several years. The subject (N=45) had to lift an object which could vary in size and weight using a precision grip. The object was transported until the information required for transport was determined. The displacement of the arm and object was recorded with a Peak Performance system. The results demonstrate three distinct stages in the development of the precision grip, from 2 to 5 years old, from 6 to 8 years old and beyond 9 years old. At 2 years of age, the peak acceleration was negatively correlated (r= -0.51) with the peak grip force during lifting. By 3 years of age, the relationship between the peak acceleration and the peak grip force during lifting became positively correlated (r=0.32) and continued to consolidate until 9 years of age. Also, starting at 6 the safety margin steadily decreases and reaches a stable value by 9 years of age. (Supported by NSERC and FCAR)

THE ROLE OF THENAR MUSCLE ACTIVITY IN THE CONTROL OF INDEX FINGER FORCE. D.J. Lalonde, M. Bilodeau and R.M. Enoka. Dept. of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH 44195

Although the control of isometric abduction force by first dorsal interosseous (FDI) has been well characterized, little is known about the contribution of thenar muscle activity to index finger force under these conditions. To address this issue, experiments were conducted to examine thenar muscle activity during maximum voluntary contractions (MVCs) and submaximal constant-force contractions (5% and 20% MVC). Surface EMG signals were recorded from the FDI, adductor pollicis (AP) and flexor pollicis brevis (FPB) of 6 young subjects. With the hand positioned so that the palmar surface rested on a table edge, the thumb was extended to about 90 degrees in the same plane or abducted by about 90 degrees so that it was in a plane perpendicular to the palmar surface. The thumb was then extended as far as 40 degrees from the midposition in the plane of movement. The thumb was used for a 657-670, 1995 during maximum voluntary contractions (MVCs) and constant-force tasks; (3) decreased MVC abduction force exerted by the index finger; and (4) no effect on the control of submaximal force (coefficient of variation). These results suggest that, while thenar muscles are biomechanically coupled with first dorsal interosseous, activity in these muscles is not related to the control of index finger abduction force under isometric conditions. However, FDI activation appears to be less than maximal with the thumb in the abducted position.

Supported by NIH grant AG 09000 to RME.

ILLUSTRATIVE MOVEMENTS EVOKED BY ENSEMBLE CUTANEOUS INPUT FROM THE HUMAN HAND. D.R. Collins. Institute of Neurophysiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2C7

Movement illusions evoked by tendon vibration have established an important muscle spindle contribution to human kinesthesia. The extent of any cutaneous contribution is unclear, though recordings from skin receptors on the hand dorsum show that they can provide detailed kinematic information (Edin & Alba, J. Neurophysiol. 53:657-670, 1985). We have now tested the hypothesis that ensemble stimulation of these receptors results in illusions of movement. Two techniques were used on naive subjects. First, electrical stimuli were delivered through an electrode array (4x4) on the dorsum of the right hand fingers. The low intensity (1.1 ± 0.7 percutaneous threshold) stimulus pulses (80 μsec) were frequency-modulated from 0-700 Hz, sinusoidally at 0.3 Hz. Illusory movements were evoked in 11/13 subjects (85%) and increased with stimulation frequency, to a maximum at 300 Hz. These typically involved perceived flexion of the metatarso-phalangeal (MCP) joint(s) during periods of increasing stimulus frequency. Secondly, mechanical stretch of the skin was used to provide a stimulus of 1-2 sec duration during natural movements. Small loops of string or squares of adhesive tape were stuck to the skin on the dorsum of the hand and fingers. These were attached to elastic bands which permitted even application of constant tension to the MCP joint. These bands caused the largest overshoot of force if it occurred prior to the onset of the force. The overshoot diminished progressively as the vibration was initiated later, with no overshoot observed when the vibration started before the hold phase. We propose that sensory information from antagonist muscle spindles contributes to an internal model of the initial state of the effector prior to the onset of force, and this information mainly is used for the feedforward control of isometric force.
755.1 ARM MOVEMENT RELATED ACTIVITY IN THE SUPERIOR COLLICULUS OF THE MONKEY DURING DIFFERENT EYE POSITIONS

The superior colliculus (SC) contains neurons which are active before and during arm movements. Thus the primate SC might be also a part of the arm control system. We wanted to study these neurons and, therefore, investigated the influence of different eye positions on the activity of the SC.

In the first task, the 'Saccade-Reach-Task' (SRT), the monkey had to perform first a saccade and a reaching movement to the target. Then it was presented on the monitor the visual target and the monkey had to remember it. In the second task, the 'Fisheye-Reach-Task' (FRT), the monkey had to hold fixation central until the reaching target. In this condition it was represented in the retina only, although the position of the target with respect to the head was the same as in the SRT. Thus, the activity of the cells was not dependent on the position of the target in retinal coordinates.

We found two types of discharges with an equal pattern of activity during movements to the same visual target in both tasks. The activity of the group of cells did not depend on the position of the target in retinal coordinates. Therefore we conclude that these cells work in the head-centered coordinate frame and not in the SC itself.

(Supported by Neurovision and Mucoum)


The purpose of this study was to determine whether cerebellar patients make errors in early phases of reaching consistent with our hypothesis that the cerebellum generates feedforward signals to adjust for interaction torques. This hypothesis suggests that cerebellar patients will exhibit systematic directional errors in the earliest phase of reaching and 2) increase the magnitude of these errors during faster reaching (increased interaction torques are generated). We also addressed whether any such errors could be due to abnormalities in relative timing patterns of muscle activity which result in the initial direction of movement.

We studied normal and cerebellar subjects reaching in a parallaxing satin under three conditions: a 'slow' condition in which seated subjects made a self-paced reach to a ball mounted on a 1 cm target 45 cm in front of the subject, a 'fast' condition in which subjects moved as fast as possible and touched any part of the 4 cm ball, and a 'fase' condition in which subjects moved as fast as possible but were not required to stop on target. Shoulder, elbow, wrist, and finger kinematics were video tapes and analyzed. EMGs from the anterior deltoid (AD), pronator deltoideus (PR), biceps (BR), and triceps (TR) were recorded on paper and digital scopes digitized. Inverse dynamics equations were used to estimate elbow and shoulder torques.

It is likely that cerebellar patients make errors in the initial direction of movement compared with controls. As the reach velocity increased, there were increased directional errors consistent with an inability to adjust for the interaction torques generated at the elbow. The EMG analysis showed that the onset of entire pattern of muscle activity was delayed but the relative timing of the two muscles active during early phases of movement (AD and BR) was normal. These findings support the idea that the cerebellum helps to initiate movement and that it may play a role in the control of interaction torques.

We speculate that the cerebellum compensates for interaction torques in the early phases of movement. (Supported by The Foundation for P.T. and NIH grant NS12777.)

757.0 SPECTRAL ANALYSIS OF CUTANEOUS MECHANORECEPTOR ENG ACTIVITY RECORDED FROM A DIGITAL NERVE IN MAN

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Activity recorded from the cutaneous afferents in the skin of the fingers is being studied to develop control signals for PNS based grasp restoration neuroprosthetics. These signals are also related to the role of cutaneous feedback in the control of fine motor movements. We have characterized a group of afferents whose receptive fields were chronically around the common digital nerve subcutaneous lateral border of the index finger in a volunteer subject with spinal paraparesis and C6 level function. A servo-controlled mechanical stimulator was used to indent the skin at predetermined rates or to produce sliding movements of a rectangular shaped, flat surface, contactor. The recorded ENG activity was rectified, band-pass filtered (14-5-Hz), sampled at 12.8-KHz and digitally stored for further analysis. Mechanical stimuli representing contactor perpendicular and tangential forces and positions were sampled at 400 Hz and also stored. The ENG data were analysed for spectral components using a PC and Fast-Fourier algorithms from Matlab software.

Thus far, it is found that the frequency spectrum associated with the neural afferent activity evoked by the loss of skin contact has a more discreet frequency spectral composition than those evoked during the initiation of skin contact. These findings are encouraging since the utility of whole nerve afferent recordings for the control of PNS neuroprostheses is dependent on our ability to distinguish the underlying mechanical events at the skin object interface purely by examining the mechanoreceptor afferent activity.

CONTROL OF POSTURE AND MOVEMENT: REACHING II

756.2 IMPAIRMENT OF VISUOMOTOR TRANSFORMATIONS IN ALZHEIMER'S DISEASE

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In planning movements to a visual target, the nervous system integrates multi-sensory information to make the spatial transformations needed to bring the hand to the desired location. Since spatial localization may be impaired in Alzheimer's disease, planning is altered in this condition. We studied patients and age-matched controls and asked 12 subjects to reach targets on a horizontal digitizing tablet from midline and lateral initial hand positions without seeing their arm. Target and cursor positions were displayed on a vertical screen in front of, or to the side of the subject. With cursor feedback, paths of both patients and controls were uniformly distributed. When the cursor was blanked during movement, controls showed directional biases varying with initial position (Ghildari et al., 1995). Neither extent nor direction varied with screen location. However, when patients moved without feedback the cursor, paths were biaised towards the location of the screen where the targets were being displayed for all initial hand position. For example, when the initial hand position was in the midline, hand paths directed forward were mapped on the screen and curved towards the midplane when the screen was also in the midline; when the screen was to the right, movements were biased to the right. For initial positions to the right of midline, paths showed a corresponding bias accordingly to the screen location.

We conclude that Alzheimer's disease impairs the ability to transform the arbitrary spatial reference frame of the computer screen into the hand-centered coordinate system normally used for motor planning.

756.4 THE USE OF PROPRIOCEPTIVE INFORMATION IN POINTING DEPENDS ON AVAILABILITY OF VISUAL INFORMATION: EVIDENCE FROM A DEAPREDENT PATIENT, E. Borkhimi, O. I.,, B. C. C. E. J. and Poziner, H., Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, Institute for Problems of Information Transmission, Russian Academy of Sciences, Moscow, 104474, Poole Hospital NHS Trust, Poole, England

Analysis of errors of healthy subjects pointing to remembered targets presented in 3D space in a darkened room and data of a subject that suffered a severe inaccuracy when pointing with vision of the moving fingertip than when pointing without any visual feedback (Borkhimi et al., 1995). However, the role of proprioception of the moving arm under these two conditions is unclear. To uncover its role, we tested a subject with a severe large-fiber sensory neuropathy with complete loss of proprioception as well as 2 other subjects. Without visual feedback, this subject's pointing accuracy was about half that of the control subjects. However, when vision of the moving fingertip was available, his 3D pointing accuracy was even better than that of the controls. It seems that proprioceptive information from the moving arm is necessary for accurate pointing without visual guidance. However, since this rehabilitated deafferented subject showed the same pointing accuracy as the controls when vision was available the proprioceptive information from the moving arm is not critical to accurate pointing, but in the context of control information it is available. Thus, this under this latter condition, vision is dominant over proprioception.

Although the accuracy of pointing in darkness was significantly different for control and deafferented subjects, the mean maximum speeds across all target locations were similar. Moreover, the same speed of pointing movements was observed in both groups for visual and visual guidance. However, the capacity to vary movement speed was not the same for control and deafferented subjects. When subjects were required to move with different speeds (fast and slow), the speed of pointing movements for deafferented subject, whereas, control subjects could change their speed 8-9 fold.

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756.5 LEARNING NON-LINEAR VISUOMOTOR MAPPINGS. J. R. Flanagan*, A. R. Rao. Queen's University, Kingston, Canada and Teachers College, Columbia University, New York, U.S.A. 

Reaching movements are characterised by roughly straight hand paths in Cartesian space. However, the issue remains whether this reflects constraints on perception or production. To address this issue, we examined two-joint planar reaching movements in which we manipulated the mapping between actual and visually perceived hand motion. In particular, we used a non-linear transformation such that straight hand paths in Cartesian space would result in curved paths in perceived space and vice versa. Under these conditions, subjects learned, after about 500 trials, to modify their movements so as to generate straight paths in perceived space. This suggests that reaching movements are planned in perceived coordinates.

In a second experiment, we tested whether and how learning this non-linear visuomotor transformation transfers to novel targets. Subjects first performed over 300 trials among 3 training targets and were then presented with novel targets. We observed strong transfer to novel targets located both within and outside the workspace defined by the training set. This indicates that subjects did not simply learn a set of transformations specific to the training targets. However, transfer was not perfect. This suggests that subjects did not learn the transferrable aspects of the visuomotor mapping. The implications of these findings for theories of trajectory formation and learning are considered.


Accurate reaching towards a visual target is initially disturbed when the visual field is displaced by prisms, but gradually recovers. To clarify whether the mechanisms of improvement are common among reaching movements with different velocities, transfer of the adaptive improvement between rapid and slow reaching movements was studied.

Four human subjects were trained to reach rapidly (<300 msec) or slowly (~5 sec) at a target that appeared to be at a random location in target space (400 mm away). Vision of the target and the hand was always blocked during the movement by liquid-crystal shutters, and allowed exactly after the index finger touched the screen. One experiment consisted of 3 sets (set 1-3) of 60 trials. In set 1, the subject wore no prisms and made 15 rapid reaching movements followed by 15 slow, or 15 slow then followed by 15 rapid. In set 2, the visual field was displaced to the right or to the left by prisms (15 diopters) and the subject was required to make reaching movements with the same velocity as in the latter half of set 1 throughout the 30 trials. Initially the subject misreached the target by about 60 mm in the direction of the visual displacement, and the errors decreased with trials down to the control level by the end of set 2. In set 3, the subjects wore no prisms and were required to reach with either velocity. The initial errors in set 3 were 51±19 mm (mean ± s.D., n=16) when the movements with the same velocity were required in both set 2 and 3. In contrast, the initial errors were only 17±16 mm (n=16) when the required velocity in set 3 was different from that in set 2. Analysis of variance revealed that the magnitude of the initial error in set 3 did not depend on the velocity. The effects were not observed when the velocity classes in set 2 and 3 were the same or different (p<0.001). These results indicate that the major part of the adaptive improvement in rapid or slow reaching movements does not transfer to the movements with the other velocity, and suggest that independent mechanisms are involved in the improvement of rapid and slow reaching movements.


One basic aspect in motor learning is the amount and type of feedback involved in the movement. Freud (1986) proposed two movement categories: type I movements are slow and under focal sensory control, whereas type II movements are fast, overlearned and under sensory field control. The transition from type I to type II movements can be considered as motor learning and may be described as a change in several parameters: reduction in movement time, reduction of the number of velocity changes during the movement and development of isochrony (decoupling of movement time and movement amplitude).

These parameters were examined in a tracking task using healthy subjects. Subjects had to track a figure consisting of straight lines with a pencil: data were recorded with a digitizing tablet. Two groups of subjects started with either the right or left hand. After twenty trials they changed the performed hand for another twenty trials. An isochrony index was computed for each trial using the movement times for different parts of the figure. The results show a learning effect in the beginning hand and a learning transfer to the contralateral hand.

The results suggest that feedback from the subject's hand movements is crucial for learning in this task, whereas feedback from the subject's visual perception of the target is less important. This is consistent with the hypothesis that learning in this type of task is driven by the subject's own actions rather than by the subject's perception of the environment.


756.8 ADAPTIVE INTERNAL MODEL OF INTRINSIC COORDINATES TRANSFORMATION DURING LEARNING OF A REACHING TASK. L. Immirzi*, M. Uno and M. Kawato. ATR Human Information Processing Research Labs, Kyoto, Japan.

Recent computational studies have proposed that the central nervous system acquires internal models from task-orientation. This transformation from task-oriented extrinsic space and intrinsic space such as joint angles to investigate acquisition of the internal model, we virtually manipulated (1/2) the angle of the elbow and magnified (3/4) the shoulder angle of human arm while they were aiming at targets. A position marker was attached to the subject's hand and its current altered position was displayed as a cursor on a CRT screen. This linear transformation in joint angles (intrinsic coordinates) corresponds to a nonlinear one between the end point plane and the screen (extrinsic coordinates). We investigated whether the subjects learn this transformation as the former (current) or the latter (screen) by manipulating the orientation of the current condition. We found that the consistent condition was significantly larger (p<0.02) than that in the inconsistent condition. Furthermore, the error in the corrective oscillation became small after they learned the transformation using the opposite arm in the same condition compared with when they learned it for the first time (p<0.001). That is, intermanual transfer of the learning effect was found in the consistent condition but not in the inconsistent condition. Results suggest that the subjects learned the transformation as a linear one in intrinsic coordinates in the consistent condition and that the central nervous system adaptively represented the transformation including intrinsic coordinates in the control of arm movements.

756.9 SELECTIVITY OF ADAPTATION IN MOTOR LEARNING. P. Gosподь*, K. Misono-Ikeb*, and E. Bink. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139. (*) Northwestern University Medical School, Chicago, IL 60611.

We studied the specificity of the adaptation to perturbations during pointing movements. We asked human subjects to execute point-to-point movements between targets arranged in a star-like fashion. The movements were planar and the targets were coupled to a manipulandum which was used both to record kinematic data and to perturb the subjects. The perturbations, which were normal to the movement and proportional to the movement velocity, significantly altered the trajectory. We tested the subjects after the perturbing forces were disconntined and observed aftereffects as a mirror image of the perturbed trajectories.

We initially trained subjects to make movements to a subset of the targets while being perturbed, and then tested them on all the other targets without applying perturbations. To test for generalisation of learning we observed the magnitude of the aftereffects in the untrained directions and found that the aftereffects decreased with distance from the trained trajectories. As a further test of the adaptation, we trained the subjects to two different perturbation amplitudes, one large and one small, in a particular arm configuration while keeping the end point trajectory the same. The subjects learned the tasks and showed aftereffects specific for the field related to the posture to which they had been exposed.

Taken together, these results indicate that the effects of motor adaptation tend to be local to the region where training has occurred. In addition, our experiments are compatible with adaptation being represented in invariant coordinates.

Acknowledgements: ORH, N00014-95-E-0073 and MCB ARM7610. FO was supported by a fellowship by SSJA.

756.10 GENERALIZATION OF ADAPTATION TO CORIOLIS FORCE PERTURBATIONS OF REACHING MOVEMENTS. J.R. Lackner* and P. Dizio. Ashton Graybiel Spatial Orientation Laboratory and Vollen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Visually open-loop reaching movements made in a rotating room are initially deviated in the direction of Coriolis force perturbations, Cₚ, generated during movements; endpoint accuracy and straight line paths are restored within ~10 reaches. Mirror-image aftereffects occur when rotation stops, revealing motor reprogramming to balance the velocity-dependent, non-contacting, inertial Cₚ perturbations. Here, we studied in seven subjects already adapted to 10 rpm rotation the effects of intensifying Cₚ by increasing reaching velocity ~1.5 times. Renewed endpoint and curvature errors in the Cₚ direction were initially generated. Subjects adapted to the new reaching speed, at the same rate as to the original one. Mirror-image aftereffects did not occur when the original reaching speed was resumed. Thus, after being exposed during rotation to the Cₚ fields generated by just two different reaching velocities, a generalized adaptation occurred such that changing reaching speed again did not affect reaching trajectory or accuracy. Supported by NAG 9-515 and NAWC 91-359-94-C-0002.
756.11  
DELAYED VISUAL FEEDBACK IN SINGLE JOINT MOVEMENTS.  
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As part of our study of the role of visual and kinesthetic information in coordination of multi-joint movement, we have studied the effect of introducing visual delays in the feedback of limb position and information during single joint movements. We studied wrist (30 deg) or elbow (70 deg) movements in a visually guided planner, flexion/extension task while the position of the other joint was fixed. EMGs were recorded from wrist and elbow flexor and extensor muscles. We introduced time delays of 100, 200, 400 and 800 ms in the visual feedback to the subject of limb position information. 
With visual delay no changes were observed in the kinematics or the EMG patterns of single joint elbow movements compared to no delay condition. In spite of the fact that the wrist position was fixed, clear plastic EMG activity occurred in both the wrist flexor or extensor muscles. In the case of single joint wrist movements, the wrist tended to move slower with short delays (200 ms) and faster with long delays (400, 800 ms) compared to the no delay conditions. These kinematic changes were reflected in the wrist EMG patterns. Clear plastic EMG activity occurred in the elbow flexor and extensor muscles in this condition where the elbow position was fixed. 
The data suggest that the CNS can more readily compensate for altered visual input during elbow movements than during wrist movements. EMG activities in muscles actuating about the non-moving joint may reflect learned programs related to compensation for or utilization of reaction forces during multi-joint movements involving the elbow and wrist.

756.13  
RELIANCE ON VISUAL FEEDBACK IN THE PERFORMANCE OF ARM AIMING MOVEMENTS FOR TWO DIFFERENT AGE GROUPS.  
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It is well documented that the elderly exhibit longer movement times than the young when performing simple aiming movements. One possible contributory factor to this slowing is that the elderly rely more on visual feedback, requiring them to move more slowly in order to process visuo-motor information. Subjects made a horizontal aiming movement with a lever through the horizontal plane towards the body midline to a target located 30 cm from the home position. When subjects moved the lever, a cursor on a computer monitor directly in front of the subjects moved by a corresponding amount. The elderly subjects were affected more than the young by removal of visual information regarding arm position, reflected by a greater increase in absolute error. Following extensive training, the young subjects exhibited reduced endpoint error when visual feedback of arm position was withheld while the elderly did not. The kinematic results also suggest that elderly rely on greater reliance on visual control when performing simple aiming movements, and that in contrast to younger adults, this reliance is not reduced with practice. 
Supported by Flinn Foundation and NINDS NS17421 grants.

756.12  
DELAYED VISUAL FEEDBACK IN A PLANAR TWO-JOINT MOVEMENT.  
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In movements involving more than one limb segment, little is known of the relative importance of the visual and proprioceptive information related to movement about the different joints. In the present study we examined movement kinematics and kinetics and related EMGs in a planar, two joint (elbow/wrist) movement task. The task required accurate positioning about both the wrist and elbow. Movement amplitudes were 30 deg (wrist) and 70 deg (elbow). We inserted temporal delays (200, 400 and 800 ms) in the visual feedback information to the subject of the position of either or both limb segments. 
Changes observed in elbow and wrist kinematics were graded with the delay. At smaller delays, movement velocity at both joints was reduced (compared to no delay conditions). As delay was increased, movement velocities increased as did agonist EMGs. At the wrist, this increase in agonist EMGs was not accompanied by change in the antagonist. Kinematic and EMG changes occurred independently of whether delay was applied to either or both limb segments. Movements around the elbow retained their accuracy despite changes in agonist EMG speed whereas wrist movement under- or overdrew the desired amplitude. With increasing delays reaction torque at the wrist increased slightly while muscle torques showed relatively large increases. 
The data suggest that altering the normal relationship between visual and proprioceptive information not only affects the kinematics of individual joints but also the coordination between them. It also suggests that when this relation is altered, movement about the proximal joint is preferentially preserved.

757.1  
RELATIONSHIP BETWEEN CORTISOL AND VERBAL MEMORY IN YOUNG ADOLESCENTS.  
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As part of a longitudinal investigation of the behavioral and psychological effects of steroid hormones during puberty, the present study examined the relationship between salivary cortisol levels and verbal memory in a sample of healthy young adolescents (12-16 yrs-old) and females (n>99). Immediate and delayed verbal memory was assessed by means of a paragraph recall task analogous to the logical memory subtest of the Wechsler Memory Scale. Saliva samples were collected on strips of filter paper at the end of the test session and cortisol levels were determined by radioimmunoassay. Salivary cortisol levels and verbal memory scores were significantly higher for females than for males (p<.05). Cortisol levels were not significantly related to immediate paragraph recall scores in either sex. However, girls with higher cortisol levels performed significantly better on delayed paragraph recall and exhibited significantly less verbal memory score decay than did girls with lower cortisol levels (p<.05). These results suggest that moderate cortisol elevation may facilitate particular aspects of cognitive performance in female adolescents.

757.2  
ASSESSMENT OF COGNITIVE DEVELOPMENT IN ADOLESCENTS BY MEANS OF NEUROPSYCHOLOGICAL TASKS.  
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Research has shown that development of the frontal lobes of the brain continues through late adolescence, in contrast to the earlier maturation of other cortical regions. Six tasks associated with performance of parietal lobe functioning (visuo-spatial closure, proprioception, graphesthesia, stick construction, relational questions, extrapersonal orientation) and 6 tasks associated with frontal lobe functioning (Road Map Direction Sense, Visual Verbal Test, Wisconsin Card Sorting Task, Stroop test, Wang's test, and personal orientation) were administered to two groups, 12 females and 36 males. These subjects were classified into 3 stages of development: prepuberal, midpuberal, and postpuberal using two pubertal assessment scales. ANOVA with age as a co- variate resulted in stage main effects for performance on 10 tasks, nine of which were associated with frontal lobe performance. This suggests that the tasks associated with frontal lobe function show more improvement with pubertal development than tasks associated with parietal lobe functioning. These findings demonstrated that neuropsychological tests which are typically used to assess cognitive effects of brain damage are extremely sensitive to developmental changes in normal cognitive function.
575.3

As a follow-up to our first study of frontal cortex activation in children with fMRI (Casey et al, 1995), we have recently collected data from 3 normal children (8 to 11 years) and 2 young adults on a go-no-go task. The task required the subject to respond to any letter but an X with 75% of the trials being targets or non-X. We have observed significant behavioral deficits in this task by children with Attention Deficit Hyperactivity Disorder. We hypothesized that this task would activate target brain regions of the prefrontal and limbic basal ganglia thalamocortical circuits assumed to be involved in inhibitory control processes. As predicted, all 3 subjects showed activation in the anterior cingulate and dorsolateral prefrontal regions and 3 of the 5 subjects showed activation in the medial orbital frontal cortex. Activation decreased as a function of time on task for both the anterior cingulate and dorsolateral prefrontal cortex. The location and magnitude of activation was similar for the children and adults.


575.4
CHILDREN'S VERSION OF THE ALTERNATIVE IMPAIRMENT INDEX: A PILOT STUDY. M. Hodges. Dept. Psych Assoc, Towson, MD 21204

Recently, the Alternative Impairment Index (AII) has been proposed as a new measure of neuropsychological impairment in adults. The AII is composed of scores derived from the Halstead-Reitan Neuropsychological Test Battery. This pilot study investigated the feasibility of a Children's Version of the AII (the AIT) for 16 children (4 Normal Controls, 3 Brain Tumors, 1 Brain Abscess, 1 Autism, 1 Encephalitis, 4 Traumatic Brain Injury, 3 Learning Disabled, 1 Behavior Problems) between the ages of 9 and 14, who have been administered the complete Halstead-Reitan Neuropsychological Test Battery for Older Children, were obtained and the Children's Version of the AII and the Children's Total Neuropsychological Deficit Scores were compared on agreement for level of severity. The results (i.e., 56% or 9/16 correct agreement) suggest weak levels of agreement.

575.5
MISMATCH NEGATIVITY INDICATES SPEECH DISCRIMINATION IN PRETERM INFANTS

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Recent studies have demonstrated auditory discrimination in infants. However, there is no direct evidence of auditory discrimination in the infants before the conceptional age of 40 weeks. Here we report first neurophysiological evidence that even preterm infants discriminate speech sounds.

Auditory event-related potentials (ERPs) were recorded to Finnish vowels /i/ and /y/, and to a boundary /y/-/y/ stimulus in preterm infants (n=11; conceptional age 30-35 weeks) with no major neurological findings. ERPs to the deviant vowel /i/ were significantly negatively displaced in relation to ERPs to the standard vowel /y/. ERPs to the boundary stimulus /y/-/y/ did not significantly differ from those to the standard vowel /y/.

The negativity to the deviant vowel /i/ resembled the auditory mismatch negativity (MMN), elicited in older children and adults by change in a repetitive auditory stimulus (even when the sounds are unattended). Judging from the scalp distribution of the present negativity, this response seems to be the preterm equivalent of the adult MMN. Thus ERPs might provide a new tool for assessing the adequacy of central auditory processing in preterm infants.

575.6
LONG TERM CHANGES IN CBF DURING COGNITION FOLLOWING SEVERE CLOSED HEAD INJURY: A PET STUDY. B.S. Kim*, J.B. Van Horn, E.R. Kapp*, G.E. Goldberg, R. Weinberger, S.J. Berman, PET Unit, CHOP, NIMH, NIH, 1044-317, 10 Center Dr., Bethesda, MD

To assess functional reorganization following closed head injury (CHI), we measured regional cerebral blood flow (rCBF) in 11 unmedicated, severely-injured patients with good recovery (male; mean age 31±6; mean time post-injury=7.3 yrs, range=4-16 yrs) during the Wisconsin Card Sorting Test (WCST) and a sensorimotor control task (WCSTom) using the oxygen-15 water positron emission tomography (PET) method. Two PET scans were performed on the same day, matched for age, sex, education, and handedness. Absolute rCBF (ml/min/100g) was determined on a pixel-by-pixel basis with a least squares method. Regions of interest were individually drawn on the subjects' conspectus PETs and applied to the rCBF data after pixel-by-pixel normalization to the global mean. Group differences in activation (WCST-WCSTom) were analyzed using paired t-tests. No differences in mean global flow for all tasks was found between WCST (rCBF=48±21±17; controls=43±24±4) and WCSTom (rCBF=49±15±8; controls=46±6±7). However, patients performed equally well as controls on all measures of the WCSTom. In contrast, patients showed lower activation in the anterior cingulate (p=0.01) but higher activation in the inferior portion of the left lower frontal gyrus (rCBF=60±44±6; controls=46±6±7). Moreover, patients performed equally well as controls on all measures of the WCSTom. In contrast, patients showed lower activation in the anterior cingulate (p=0.01) but higher activation in the inferior portion of the left lower frontal gyrus (rCBF=60±44±6; controls=46±6±7).

575.7
PRE- AND POST-SURGICAL ASSESSMENT OF VISUAL DISCRIMINATION FOLLOWING HEMISPHERECTOMY

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Studies of hemispherectomized patients have often reported the presence of residual visual abilities in the blind field of these patients. These results, however, have often been criticized. The present study aimed at further verifying the presence of such “blind-sight” by comparing the pre- and post-operative performance of a patient subjected to a variety of sensory tests. The patient, A.M., suffered from intractable epilepsy since childhood. She had her first seizure at the age of 2.5 years. An EEG carried out at the time showed epileptic activity in the central temporal regions of the right hemisphere. The epileptic activity remained unchanged until her hemispherectomy at age 17 (1993). The first set of studies was aimed at evaluating the patient’s ability to detect and localize stationary and moving visual targets. In the second set of experiments, color, form and letter discrimination was also assessed. In the localization experiment, A.M. was significantly less precise than controls in the left hemifield before surgery although she could detect the presence of the visual stimulus in that field. After surgery, she no longer detected any light stimulus in her left visual field. As pertains to color, form and letter discrimination, while A.M. consistently made errors in both hemifields before surgery, her performance improved postoperatively, her errors being confined to left hemifield. A.M. performed at chance whenever visual stimuli were presented in her left field. Overall, these results show that, in a patient with longstanding hemispheric epileptic dysfunctions, the hemispherectomy served as an improvement of the visual abilities of the residual hemisphere. Our results, however, do not support previous findings of the “blind-sight” phenomenon after hemispherectomy.

575.8
EVENT-RELATED BRAIN POTENTIALS (ERPS) INDICATE THAT BLINNNESS WITH LATE ONSET AFFECTS AUDITORY PROCESSING IN HUMANS. K. Aiba*, T. Kajikawi, A. Lehtioksi, A. Leinonen, and R. Naathanen Cognitive Psychophysiology Research Unit, Department of Psychology, FIN-00014 University of Helsinki, Finland.

Our previous results have shown that blindness with an onset during the first years of life affects the N2 component (peak latency about 200 ms from stimulus onset) of ERP associated with auditory discrimination, the N2 being reduced in early blind adults than in sighted controls. These results, supported by magnetoencephalographic source localization, suggest that in early blind humans, posterior cortical regions involved in vision would participate in auditory processing. In the present study, ERPs to repetitive 60 Hz standard tones and to 600 Hz deviant tones occurring infrequently among standard tones were recorded at 23 scalp sites from 8 sighted subjects (age 21-32 yrs) and from 16 blind subjects (age 21-35 yrs). In 8 of the blind, the blinness caused by a peripheral visual deficit had started at the age of 12 or later, and in the other 8, before the age of 2. When the subjects’ task was to count the number of deviant tones in the tone sequence, the N2 to these deviant tones was in the early and late blind subjects distributed on the scalp posteriorly. However, in the controls, this suggests that even blindness with a late onset may result in participation of visual brain areas in auditory processing.
T57.11

INFLUENCE OF ALCOHOL ON FRONTAL MIDLIME THETA ACTIVITY S. J. Laakka*, T. Jarvelisto
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After the discovery of the relationship between the human EEG theta rhythm (4 - 7 Hz) on the frontal area and problem solving task (Arellano & Schwab, 1950) there have been studies indicating a positive correlation between the theta activity and the level of performance. However, an increase of theta was reported also with alcohol that is usually associated with deterioration of performance. The occurrence of theta activity was studied in a simulated driving task. Two independent decisions had to be made at two crossroads guided by two traffic signs. The subject had to find the correct way to drive a car through a set of roads in a computer game. Feedback about quality of performance was given after each trial. Each trial lasted about 10 s with a pause of 2 s between trials. The EEG was recorded from Fz. Theta activity was analysed during seven consecutive sections (160 ms) of the game. The results showed that successful behaviour produced more theta than unsuccessful behaviour. Alcohol significantly increased theta activity. Theta activity was enhanced after the second traffic sign and the difference between the correct and incorrect road selections disappeared. The results may be related to the general relaxing effect of alcohol as well as to the deterioration of the estimation of performance by alcohol.

T57.13

PATTERN OF COGNITIVE DEFICITS IN SUBJECTS WITH PARKINSON'S DISEASE AND MULTIPLE SCLEROSIS. M. Rost, R.E. White, R. A. Connelly*, Department of Psychology, Boston University, Boston MA 02215 and Department of Veterans Affairs Medical Center, Boston MA 02130.

The concept of "subcortical dementia" has been used to describe a pattern of cognitive impairments common to several disorders in which the initial or principal (though not exclusive) locus of pathology is one or more subcortical structures. The pattern comprises deficits in memory, visuospatial functions, handwriting, language, and memory. The PD and MS groups were impaired on tests of psychomotor performance relative to the control group, and the MS group was additionally impaired on several measures of reaction time. The PD and MS groups differed in regard to which psychomotor tests elicited performance below that of the control group. The differing patterns of performance raise issues concerning the concept of subcortical dementia.

T57.10

ASSOCIATION OF TEMPORAL PROCESSING AS MEASURED WITH AUDITORY ORDER THRESHOLD AND PERSONAL TEMPO IN PATIENTS WITH TEMPORAL LOBE DAMAGE. S. Soni, M. Xie, A. M. Witte and E. Poppel. Inst. Med. Psychol., 80366 Munich Univ., and Research Center (KFA), 52425 Jülich, Germany.

It has been demonstrated some time ago that patients with left hemisphere injuries show considerably prolonged temporal order thresholds (OT) which represent the time needed for event identification in a sequence. Instead of normal values of approx. 30 ms, latency results in a patient. In a new study we replicated this finding and discovered in addition that the disturbance of temporal processing is limited to patients with aphasic speech problems. Patients with injuries of the left hemisphere as well as patients with anterior or posterior right hemisphere lesions showed no such effects. Interestingly, a similar dissociation of functional distance is observed with a qualitatively different experimental paradigm. The patients were asked to tap with their index finger a response key in a regular and convenient way ("personal tempo"). With this experiment the following variables can be analyzed independently: 1. key touching time (KTT), i.e. the interval between the end of an agonistic and the beginning of an antagonistic finger movement, 2. pause interval (PI), i.e. the interval between successive KTTs. Whereas KTT appears to be closely linked to motor execution, PI appears to reflect a more central cognitive component, namely an individually chosen tempo of movement. For both these variables a similar result was observed as for OT. Only patients with aphasic problems showed temporal alterations (a significant prolongation of KTT and PI) whereas all the other patient groups compared to a healthy control group appeared to be unaffected. Thus, different domains of temporal processing (high frequency sensory decoding, motor execution, and voluntary temporal control) appear to be closely associated. (Supported by DFG and BMBF)

T57.14

METABOLIC AND ANATOMIC IMAGING OF THE MEDITAL TEMPORAL LOBES CAN LATERALIZE TEMPORAL LOBE EPILEPSY BUT COMMONLY-USED VERBAL AND NONVERBAL MEMORY TESTS CANNOT. Z. Caramanos*, F. Cendes, M.A. Thomas, D. L. Arndt, and M. Patrides

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We compared, in 36 unselected temporal lobe epilepsy (TLE) patients (25 left, 11 right), the abilities of commonly-used memory tests and quantitative neuroimaging techniques to lateralize the dominant cerebral seizure origin as determined by extensive clinical electroencephalographic (EEG) recordings. Patients' delayed recall of the Rey Auditory Verbal Learning Task, the Rey Complex Figure, and the Wechsler Memory Scale (Logical Memory, Associate Learning, and Visual Reproduction Tests) was compared with their hippocampal volumes, as well as with their medial/TL levels of the neuronal marker N-acetylaspartate as determined by the proton MR spectroscopy. Although results on the neuroimaging measures were highly intercorrelated, they were not related to the patients' memory scores. Moreover, whereas patients with left and right TL EEG differences showed an effect on their memory tests, no significant lateralization was observed. Furthermore, whereas neuronal imaging models were able to explain the patients' EEG disturbance with 100% accuracy, models based on memory testing results were not much better than base rate information alone (i.e. 27 versus 25 correct).

These findings suggest that whereas these anatomic and metabolic imaging techniques are useful in the presurgical evaluation of TLE, these widely used tests of verbal and nonverbal memory are not. Nevertheless, these and other neuropsychological measures provide important information regarding the patients' cognitive abilities and should be included in any thorough clinical evaluation of TLE.
REGIONAL CEREBRAL BLOOD FLOW DURING REPEATED EXPOSURE TO A VIGILANCE TASK IN ADULTS WITH ATTENTION DEPARTMENT HYPERACTIVITY DISORDER. H.B. Schneck, J.B. Schneck, T. Hoffman, and J. Tuiten. Dept. of Psychiatry, Radiology, Neurology, Emory PET Center, Emory University, Atlanta, GA 30322.

There is increasing evidence that Attention Deficit Hyperactivity Disorder (ADHD) has a neurobiologic basis. Behaviorally these symptoms increase with repeated exposure to a task. Using the Paced Auditory Serial Addition Task (PASAT), an auditory vigilance task, we examined changes in regional Cerebral Blood Flow (rCBF) in 4 adult right-handed males with ADHD and 5 normal right-handed male controls. Subjects were scanned in two consecutive days with 2 testing, normal 2 and 2 PASAT conditions per day, each associated with “interobserver” administration. In this ongoing study, different task related increases and decreases in rCBF between the groups emerged. So far, the ADHD group showed significant activations in the right visual cortex and visual association cortex during the PASAT compared to the control task (p<.01). Control subjects showed activations in the right parietal and left inferior frontal regions during PASAT (p<.01). Changes in rCBF with repeated task exposure, were assessed by subtracting images acquired on Day 1 during the PASAT from Day 2. In ADHD subjects there were increases in rCBF in the left, superior temporal lobe from Day 1 to Day 2 (p<.01); however there were no significant decreases in rCBF from Day 1 to Day 2. Control subjects showed activations in the inferior frontal lobes, the superior parietal lobes, and the left gyrus rectus from Day 1 to Day 2 (p<.01). Control subjects also showed decreases in rCBF from Day 1 to Day 2 in the left temporal lobe and the cerebellum (p<.01). These results suggest that different brain regions, and perhaps behavioral strategies, are used by ADHD adults to perform vigilance tasks than adults without ADHD. Furthermore, the lack of decreases in rCBF seen in ADHD subjects with repeated PASAT exposure suggests a sustained processing demand, that is diminished with practice in control subjects.


Both schizophrenia and temporal lobe epilepsy (TLE) are thought to arise from temporal lobe damage early in development. MRI studies have demonstrated reduced superior temporal gyrus (STG) volume in schizophrenia, and reduced medial temporal lobe volume in epilepsy. To determine if reduced volume of the STG is specific to schizophrenia, we compared the left and right STG of TLE patients (13 left focus, 7 M and F, mean age: 27.5 yrs; 9 right focus, 5 M and F, mean age: 35.0 yrs) with that of schizophrenia patients (11 M and F, mean age: 32.6 yrs). Volume/volume analyses were performed on 3D MR images reformatted into 1mm coronal sections. The STG volume was measured between Mm anterior and Sm posterior to the anterior comissure (Barba, et al., 1991). There were no differences in the STG volume among any of the groups. There was a significant and positive relationship between right STG size and IQ in the schizophrenia patients. In contrast, in the left focus epilepsy patients, IQ was significantly related to the size of the left STG (F=6.61, p<.05). There was no relationship of IQ to the size of either STG in the right focus group. Differences in these brain-behavior relationships may be due to differences in the timing and location of early brain damage. Prenatal damage in schizophrenia may be early enough to allow, or massive enough to force, a shift of cognitive function to the right hemisphere. (Supported in part by the VA Medical Research Service and NS 93211)

SPARED FACE PROCESSING IN WILLIAMS SYNDROME: NEW PERSPECTIVES ON BRAIN-BEHAVIOR LINKS IN A GENETICALLY-BASED SYNDROME. M. Rouxen, W. Jones, & U. Belling, Laboratory for Cognitive Neuroscience, The Salk Institute, La Jolla, CA 92037.

Williams syndrome (WS) is a genetic syndrome of neurodevelopmental anomaly that involves mental retardation but presents a remarkable juxtaposition of impaired and intact mental capacities. Most prominently, linguistic functioning is preserved while problem solving and visual and verbal based cognition are impaired.

Good face processing ability is also characteristic of WS, providing evidence of asymmetry in cognition functioning within visual brain structures. We report here on performance of WS adolescents on three paradigms involving distinct aspects of face processing: Benton Face Recognition (Discrimination of unfamiliar faces); Warrington Recognition Memory for Faces (immediate recall of unfamiliar faces); and Mooney Face Classification (Perception of faces from uncloned contours). WS subjects performed significantly better than 40 normal controls on all three of these measures. Moreover, the WS subjects were indistinguishable from normal chronological age-matched subjects on the Benton faces task. Strong, significant inter-task associations exist among the face processing tasks; in contrast, no significant inter-task associations exist between the face processing tasks and WISC-R IQ, or between face processing and visual processing tasks such as a non-faces closure task (KABC Gestalt Closure), or between face processing and indices of language ability such as comprehension of grammatical forms.

Brain morphology shows an intriguing link with face processing in WMS (Jones, Rouxen, Hickok, Jerimann, & Belling, Neurosciences Abstracts, this issue). In vivo MRI data from nine WMS adolescents reveal a strong correlation between morphology on Benton Faces and volume of gray matter in inferior posterior medial cortex, normalized by total supratentorial volume (r=.89, p<.001). Warrington Recognition Memory for Faces and Mooney Face Classification correlate also very strongly with normalized IPMCG volume. These results are consistent with data from prosopagnosics on brain loci important for face processing and provide new data relevant to the neural systems underlying specific aspects of behavior.

PRINT EXPOSURE IN DYSLEXIC ADULTS: EVIDENCE FOR AN INDEPENDENT PREDICTOR OF KNOWLEDGE. J.G. Frey, Department of Psychology, Loyola Marymount University, Los Angeles CA 90045.

The clinical neuropsychological literature defines dyslexia as an impaired ability to read and comprehend written or printed words, but the etiological role of language deficits in this disorder has been a matter of considerable debate. Dyslexic children frequently have difficulties with phonological processing (e.g., Mann, 1993), but linguistic deficits are not always found in dyslexics (e.g., Aaron, Olsen, & Baker, 1985; Cotheth, 1983). Stanovich and his colleagues have shown that print exposure may be an independent predictor of knowledge in normal readers with varying degrees of reading comprehension ability (e.g., Stanovich & Cunningham, 1993). Since knowledge bases play an integral role in skilled reading (Kintsch, 1983), print exposure may be an important variable to control when studying populations with reading impairments. In order to investigate these issues, dyslexic adults (18-25 yrs) who had graduated from high school and were attending college were matched by age, gender, and cognitive ability with normal readers. Linguistic, cognitive and reading skills, including phonological awareness and print exposure, were tested in the subjects. Dyslexics did not differ in phonological skills from normal readers. However, dyslexics differed significantly from normal readers in print exposure. Multivariate statistical analyses revealed that print exposure was a significant predictor of knowledge, independent of cognitive ability. These results suggest that print exposure may play an important role in cognitive processing and should be controlled in studies of dyslexic subjects.

SUPPORTED BY A LOYOLA MARYMOUNT UNIVERSITY FACULTY RESEARCH GRANT.
758.1 MATCHING PURSUIT ON ERP ANALYSIS J.J. Allen, B. Shen, J.C. Williams and J.B. Ainger* Dep. of Psychology, University of Arizona, Tucson, AZ 85721

Obtaining averaged event-related potentials (ERPs) requires more repeated presentations of stimuli or the use of single trial methods that are brain-specific and thus imperfect. For single trial events, traditional analysis techniques (spectral analysis and cross-correlation) often fail to extract information from noisy signals reliably. By averaging a large number of trials, the signal-to-noise ratio of temporal processes in ERPs may be enhanced, but at the price of losing frequency information. A recently developed technique, matching pursuit analysis, offers flexible decompositions of signal components localized in both time and frequency domains (Mallat and Zhang, 1993). We found that this technique could extract both temporal and frequency information from noisy single trial ERPs. Averaging as few as several ERPs over the transformed domain provided distinct temporal frequency patterns that corresponded to the classic P3 component of the ERP in a memory-assessment task. Random noise and systematic artifacts of ERPs could be rejected by simply subtracting appropriate time-frequency atoms. For us, this technique not only provided a more efficient and precise tool for analyzing averaged ERPs, but also suggested a new approach to the analysis of single trial events.

758.2 POSSIBLE FRONTAL LOBE CONTRIBUTION TO NEGATIVE REPETITION PRIMING S. Yamagata, S. Yamaguchi* and S. Kobayashi
Dep. of Internal Medicine III, Shimane Med. Univ., Izumo, 693, Japan

Several lines of evidence have shown that the repetition priming effect is sensitive to the direction of attention. On the other hand, one recent study confirmed an inverse repetition effect to words at the unattended location. We studied the positive and negative repetition priming effects on attended and unattended words during a lexical decision task and examined the contributions on those effects using topographic analysis of event-related evoked potentials (ERPs). Subjects were asked to focus their attention to a word (vertically oriented Kanji letters) presented in one visual field and ignore the word in the opposite field during a task of detecting a non-word in the attended field. The field attended was informed by an arrow precise and word color. The identical word appeared sequentially 56 times at the attended and unattended visual field respectively at random. A positive ERP deflection to repeated words appeared when the sequential words were presented in the attended location. This repetition effect was greater over the posterior scalp sites contralateral to the attended field. Non-attended repeated words generated a negative-going shift over the left frontal scalp sites regardless of the field of word presentation. These results suggest that the frontal lobe may contribute to the active inhibition of implicit word processing within the unattended channel.

758.3 FUNCTIONAL TOPOGRAPHY DURING PROCEDURAL LEARNING STUDIED WITH EVENT-RELATED DESEQUENCING MAPPING (PRELIMINARY FINDING) P. Zhong, C. Toro, J. Graffman, P. Mangun*, L. Leocani, M. P. Dejer, J. Womersley*, M. Halliet, National Institute of Health, NINDS, Human Motor Control Section, Medical Neurology Branch, Bethesda, MD 20892, USA

To explore the role of the human motor cortex associated with the development of implicit and explicit knowledge, we studied event-related desequENCing (ERD) as an indicator of localized brain activation during a visual motor task. EEG signals were recorded from 3 right-handed subjects as they performed a variation of the serial reaction time task (SRTT). ERD was calculated within the alpha (8-12 Hz) band from EEG recording of 29 scalp locations from -1 to 1 cm around to the correct keypresses. During the data collection, all subjects developed implicit knowledge of the test sequence, which was reflected by diminishing response time and generation of explicit knowledge. ERD maps revealed localized alpha power reactivity over the contralateral sensorimotor hand area. The area of power reactivity became progressively larger until explicit knowledge was reached, after which the power spectra showed a declining power. These electrophysiological findings are in support of our previous results demonstrating the rapid functional plasticity of cortical motor outputs associated with procedural learning and with transfer of knowledge from an implicit to explicit state.

758.4 EVENT-RELATED POTENTIAL (ERP) CORRELATES OF TARGET DETECTION: EFFECTS OF TASK DIFFERENCES L.K.G. fastt and A.W. Faszbach
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Ten subjects completed two visual "oddball" tasks involving identical stimuli; random length sequences of 3 to 9 individually presented digits. Target sets (3 digits or 3 item letter-digit sets) were presented between series in the detect condition. Subjects were asked to selectively respond to the 3 digit sets. In the detect+compare condition, subjects selectively responded to 3 number targets that matched the last 3 individual numbers presented. Error rates were similar across conditions.

ERPs elicited by target stimuli recorded from 4 midline scalp sites (Fz, Cz, Pz, Oz) included two major positive-going peaks (approximately 200 and 375 ms post-stimulus). Amplitudes of variance failed to support main effects of condition upon latencies. The condition of condition on amplitude just reached significance (p = 0.049) for the earlier but not the later peak (P300). This lack of amplitude and latency differences of the P300-like peak suggests insensitivity to considerable differences in task requirements. P300 may not obliterate the time course of ERP responses involved in more general processes that proceed in parallel with, but independent of, divergent processes in distinct tasks. Digital ERP subtraction (detect-detect + compare) revealed ERP features (Fig. 1) associated with comparison of working memory contents to external stimuli.

758.5 HEBIAN INDUCTION OF AUDITORY CORTICAL RECEPTIVE FIELD PLASTICITY: EFFECT OF NUMBER OF TRIALS & CORTICAL STAGE Scott J. Oyster* and Norman M. Weisberger, CNLM and Dep. Psychology, UC Irvine, CA

Receptive fields (RF) plasticity in the adult auditory cortex (Aud Cx) has been observed following hearing loss (Wellerger et al., 1980, CNA). We are investigating induction mechanisms of such plasticity, and have focused on Hcbian rules, because they have been implicated previously in neuroplasticity (e.g. Study of King, 1980, J. Neurobiol). A preliminary report from our lab indicated that for most cells in Aud Cx, a "Hebian" treatment (Stair, 1987) resulted in a change in RF plasticity (see Avendano et al., 1993). Since that report, we have studied several factors that seem to affect the probability of observing RF plasticity following a "Hcbian" treatment. These include number of pairing trials and cortical RF size.

Procedure: In mouse anesthetized Guinea pigs, first, a single postsham (posttemporal) Aud Cx cell was isolated with a KCl-filled microelectrode. During a baseline period, two Aud stimuli of differing frequency were presented, and paired with different sets of presynaptic afferents for the recorded cell. The response of the posynaptic cell to these two stimuli were recorded, and it was observed if the second stimulus treatment was imposed in one of the Aud stimuli (Cs) was paired with excitatory current to the posynaptic cell, to enhance the presentness of the previous response. The other Aud stimulus (Cc) was paired with inhibitory post-synaptic current. After 60 pairings, the response of each cell was tested for the number of pairings made in the treatments. Only 50% (16%) of cells were significantly controlled during the treatments. Each cell was significantly more than the number of trials on plasticity. EEG was also recorded.

Results: No significant growth was observed in the relative response of the posynaptic cell to the two Aud stimuli (Cs/Cc−Cs/Cc) for each trial in each period. The distributions of these relative scores were compared between experimental groups (P<0.05). All cells (28/28) were significantly controlled in the treatments. Only 50% (16%) of cells had significant relative increases for the Cs after the first treatment block compared to baseline. However, there were much larger effects after the 2nd treatment block. The relative increases for the Cs at the first treatment block compared to baseline. However, there were much larger effects after the 2nd treatment block. The relative increases for the Cs at the first treatment block compared to baseline. However, there were much larger effects after the 2nd treatment block. The relative increases for the Cs at the first treatment block compared to baseline. However, there were much larger effects after the 2nd treatment block. The relative increases for the Cs at the first treatment block compared to baseline. However, there were much larger effects after the 2nd treatment block.

758.6 THE P3-LIKE LONG-LATENCY COMPONENT IN RATS: IS IT A HIPPOCAMPAL THETA WAVE? J. BrankS* and T. Seidenbocher, Inst. of Physiology II, Heinrich-Heine-University, Dusseldorf and Institute of Neurobiology, Magdeburg, Germany

Long-latency components of event-related potentials (P3 or P300) correlate with the ability of subjects to detect and process unexpected, novel or task relevant events. Several conditions were recorded in the neocortex and hippocampus of rats performing an auditory discrimination task, similar to the oddball paradigm used in human psychophysiological experiments. In anesthetized rats and surgical electrodes were implanted at several neurocortical regions and the hippocampus. After recovery from surgery rats were trained to discriminate two auditory signals, a frequent irrelevant tone (T1) and a rare tone (T2) related to water reward. In response to T2 but not T1, P3-like components with a mean latency of 274 ms (range: 244 to 305) and a mean amplitude of 67 μV (range: 24 to 158) were recorded from the surface of the neocortex. The largest amplitudes were found in the anterior part of area OC2M situated above the hippocampal CA1 region. Powerspectra of differences between responses to T2 and T1 revealed peaks in the theta range (4-12 Hz) maximal at area OC2M. The amplitude increased with depth to a maximum of 298 μV in stratum oriens of the CA1. A polarity reversal occurred at the pyramidal cell layer (-175 μV). The largest negative amplitude was found in stratum radiatum (+304 μV). It is suggested that the P3-like component in rats corresponds to a theta wave observed in the hippocampal theta cycles. This work was supported by the Deutsche Forschungsgemeinschaft grant Ba 1289/1-1.
LEARNING AND MEMORY: PHYSIOLOGY III

758.7

N400-LIKE EVOKED POTENTIALS IN THE MACAQUE HIPPOCAMPUS


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Several anatomical studies of the P300 evoked potential but not ERP correlates of cognitive EEGs of the N400-family have received scant attention. We recorded intracranial VEPs (per-implant skull screw reference) from stereotaxic probes implanted in a macaque monkey (M. radiata). The task required the monkey to distinguish familiar from unfamiliar faces. Major components from a right hippocampal contact (coordinates A12.1, R11.27: atlas of Sze and Cowan) were recorded in a delayed block design (15 trials, block 1122±19). N194 (5.3), N266 (14.0). Here amplitudes (μV) of the two largest components in the hippocampus vs. values at immediately superior and inferior sites:

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<th>Type</th>
<th>N194</th>
<th>N266</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>superficial (D25)</td>
<td>90.7</td>
<td>10.8</td>
</tr>
<tr>
<td>deep (D29)</td>
<td>10.6</td>
<td>7.4</td>
</tr>
</tbody>
</table>

The inversion of N194 and the steep voltage gradients of both components suggest that the two are generated locally in the hippocampus. Thus, local field potentials do not occur in the monkey hippocampus in response to the same stimuli and in the same task that evoke large N130 and N430 components in the human hippocampus. While precise component homologies will require further work with additional tasks and in other subjects, monkey components appear generally to be earlier than in humans.

Supported by VA Merit Review and USPHS NS18741

758.8

NEURONAL ACTIVITIES IN MEDIAL PREHOMOR AREA OF MONKEY DURING LEARNING OF SEQUENTIAL MOVEMENTS.

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To investigate where and how the memory for sequential movements might be represented in the brain, we trained a macaque monkey to perform a sequential button press task, “2x5 task”. On pressing a home key, two of 16 (4 × 4 LED buttons called ‘set’ zone) were illuminated randomly. The monkey had to press in a predetermined order which he had to find out by trial-and-error. A total of 10 sets (a “hypertrip” was presented in a fixed order for completion of a trial. A hypertrip was a repeated pattern of sequences which were repeated a new hypertrip during a block of experiment. 14 hypertrips were assigned to “learned hypertrips” which had been learned daily so that the animal could perform them with few errors.

We recorded spike activities of 154 single neurons in the mediodorsal area as the monkey was performing “learned” or “new” hypertrips. 68 cells (44%) showed differential activities between learned hypertrips and new hypertrips. Among them, 51 cells showed learning dependent changes within a block of experiments during which the monkey repeated a new hypertrip: (1) 30 cells initially did not show task-related activities, but as learning proceeded, activities appeared that were related to hand movements of particular translations or combinations of sequences; (2) 11 cells showed strong activities for new hypertrips which became weaker as learning took place. These changes of activation pattern occurred for each new sequence separately. These results suggest that mediodorsal area participates in short-term and long-term learning of sequential movements.

758.9

REWARD-DEPENDENT ACTIVITY OF DIFFERENTIAL DELAY NEURONS IN THE PRIMATE PRERECENTOR CORTEX.

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In the delayed response task (DR), “Differential Delay Neurons” (DDN) are observed in the primate prefrontal cortex (PFC), which show differential activity between right and left trials during the delay period. The delay time was considered to be related to the spatial working memory. In the last year’s meeting, we reported reward-dependent delay-related activity in the PFC neurons. Here we report the activity of DDN in various reward situations.

Three monkeys were trained on the DR using several kinds of food and liquid. They received the same reward as for a block of 30 – 50 trials. On the indirect ‘method situation’ position of a red light, and on the direct ‘method situation’ position of reward itself (food reward only), indicated the correct side.

Many DDN were found to behave in different ways between Direct and Indirect and/or between food and liquid reward and/or among different food and/or liquid reward. Showings, showings of different patterns of spatial specificity during the delay period. Among them were some such DDN which showed the spatial specificity either on Direct or on Indirect method, situation or either on liquid or on food reward situation.

The results indicate DDN could be involved in retaining the difference of reward situation during the delay period related to retaining spatial information and that the single PFC neuron could be involved in retaining more than one kind of information at the same time.

758.10

DISSOCIATION OF MOVEMENT RELATED NEURONAL ACTIVITY CINGULATE CORTEX IN MONKEYS PERFORMING OCULOMOTOR AND MANUAL DELAYED RESPONSE TASKS.

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We examined, in two monkeys performing both oculomotor and manual delayed response tasks (ODR and MDR resp.), neuronal activity related to performing both tasks. The goal was to determine whether single neurons were involved in the MDR task and whether the same or different neurons were engaged in the oculomotor and skeleton motor movements. As in the ODR task delay period activity was recorded also during the MDR task, and was not spatially tuned. The results indicate that neurons in pCC are nonspecific with respect to spatial mnemonic information; rather, their activity is related to preparation and execution of movements.

758.11


It has been shown that the anterior cingulate (AC) cortex is involved not only in learning, but also in behavioral manifestation. In the present study, single neuron activity was recorded from the AC during behavior based on discrimination of rewarding, aversive, and neutral objects. Of 313 neurons recorded from the AC, 63 responded in one or more phases of the task. Of these, 23 were found to show spatial sensitivities (differential, 19; nondifferential, 7). These 19 responded to rewarding (5), aversive (6), or reward and aversive but not neutral objects (8). Responses of 5 neurons that responded to aversive objects were rapidly suppressed by associating the aversive objects with reward (reversal). Of 15 neurons that were active in the bar pressing phase, 5 differentiated bar pressing to avoid shock from bar pressing to obtain reward. Responses of 4 neurons were anticipatory, with gradually increasing activity between the start tone and a visual stimulus (object). There were topographic distributions of these responsive neurons in the AC. After differential bar press-related neurons were located from the anterior to posterior portion of the AC respectively. The results suggest that processed and transformed sensory command from the anterior to the posterior portions of the AC.

758.12

PARIETAL UNIT RESPONSES IN A CROSS-MODAL (VISUO-HAPTIC) DELAY TASK. Yong-Di Zhou and Jordan M. Foster

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The purpose of this research is to explore the reactivity of somatosensory cortical cells to visual stimuli and their role in the mnemonic retention of those stimuli for a prospective memory choice. Monkeys were trained to perform a visuo-haptic task with a forced delay between a visual stimulus and a tactile choice appropriate to it. Each trial consisted of the following: (1) 5 sec visual cue; (2) black and white pattern of vertical or horizontal stripes; (2) delay of 10-20 sec; (3) click signalling accessibility of two parallel rods, one with horizontal and the other with vertical ridges on the surface; (4) tactile comparison. The pCC neurons (pull) of rod with ridges in the same direction of the stripes of the icon. Correct choice was rewarded with liquid reinforcement. Icon and relative rod position were changed at random from trial to trial in the vertical and horizontal condition. These results suggest that neurons in pCC are responsive to sustained visual stimuli, i.e., of more than a single direction and the other. They are associated with sustained visual stimuli, i.e., of more than a single direction and the other. They are associated with sustained visual stimuli, i.e., of more than a single direction and the other. They are associated with sustained visual stimuli, i.e., of more than a single direction and the other. They are associated with sustained visual stimuli, i.e., of more than a single direction and the other. They are associated with sustained visual stimuli, i.e., of more than a single direction. 
578.13

INHIBITION OF LOCUS COERULEUS NEURONS BY SPONTANEOUS AND INDUCED FRONTAL CORTEX ACTIVITY
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Given the neurotransmitter action of norepinephrine throughout the forebrain, identification of the neurones in the lateral aspect of the locus coeruleus (LC) cell body field have important implications for our understanding of the role of this system in many aspects of information processing. Recent evidence has shown that a small population of neurones in the medial precentral region of frontal cortex (FC) of the rat can be antidromically driven by LC stimulation; suppression of activity in these cells results in an enhancement of tonic LC activity (Sara & Hervé-Minnié, PNAS, in press). The present study examined further the influence of FC activity on neurones in ketamine-anesthetised rats. Phasic LC unit responses to FC activity were examined using two methods. 1) Spontaneously active, multiple single-units were recorded simultaneously from FC and LC. Cross-correlation analysis examining LC unit activity as a function of FC unit activity, frequently showed a transient suppression of LC firing 25-50 ms after the FC unit discharge. 2) Patterned electrical stimulation of the FC produced a transient suppression of LC activity, often followed by enhanced discharge at stimulus offset. Stimulation of the FC outside of the medial precentral region produced no, or much less, suppression. Together these data show, in addition to the previously demonstrated tonic influence, a phasic influence of a circumscribed region of FC on LC activity.

578.15

INVOLVEMENT OF GABAERGIC PROJECTIONS IN SYNCHRONOUS OSCILLATIONS IN CULTURED CORTICAL NEURON NETWORKS FURING: BICUCULLINE EFFECTS FREQUENCY OF OSCILLATION
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Synchronous oscillation of neuronal activities has been suggested to be important for sustaining firing to re-inforce activity-dependent changes of synaptic plasticity in memory circuits (Kobayashi et al SocNeurosci Abstr 991). Here we find that dissected rat cortical neurones make networks with high synchronous convergence in culture and that the networks fir spontaneously in synchronous oscillation (Kobayashi et al SocNeurosci Abstr 993). The existence of GABAergic neurons and synapses in the culture was investigated by immunocytochemistry. After fixation, approximately 10-15% of neurones were stained with both anti-GABA antibody and with Anti-GAD antibody. The proportion of GABAAergic neurones in the primary culture is thus very similar to that of rat visual cortex in vivo. Anti-GAD also stained many small dots around neurones and their dendrites. When double-staining with anti-synaptophysin antibody was carried out, many small dots of synaptophysin staining were also stained with GAD antibody, indicating existence of abundant GABAergic terminals. Since inhibitory postynaptic currents are also found in some neurones in the network (Robinson et al J. Neurophysiol. 70, 1606,1993), the effects of a GABA agonist on the synchronous oscillation of bursting activities were observed using furca-2 multi-site Ca fluorescence. Application of bicuculline decreased the frequency of synchronous oscillations suggesting a contribution of GABAergic input to the network activity.

578.17

Basalofutal amygdaloid neuron responses in rats performing a spatial maze task
W.E. Pratt1, S.S. V. Mizumori, Dept. Psychol., University of Utah, Salt Lake City, UT 84112

Much research involving the nucleus accumbens (NA) has suggested that it is a crucial site for the modulation of reward and spatial learning. Previously, we have reported the presence of cells with reward and spatial activity correlates within the NA (Lavoe & Mizumori, 1994). The pathway from the basolateral nucleus of the amygdala (BLA) to the NA is probable important in the conduction of reward information. The present study investigated information encoding within the BLA which could then pass to NA neurones.

Single units in the BLA were recorded while animals performed a spatial memory task on an eight arm radial maze. Four arms consistently had low reward (1 dr. chocolate milk) and four contain a high reward (5 drops chocolate milk). Initial results (n= 43 cells, 1 rat, 2 passes in each of 2 hemispheres) indicate a variety of behavioral correlates. 14% of cells displayed location specificity, 30% showed an increased firing rate at least 50% (range: 25-77%) when traveling outbound from the center of the maze relative to traveling inbound relative to the outbound rate (24-62% increase). 9% of cells decreased their rate 50% at the end of arm, with a corresponding increase once movement resumed. Three cells showed possible reward correlates; one fired only at the end of arms during trials, and the other two either showed a 50% increase or decrease which did not appear movement-related.

These data, while not proving or disproving a role for the BLA in reward-related processes, suggest that the amygdala may encode more spatially-related forms of information than was previously imagined. These data illustrate for the first time the heterogeneous nature of information coded in the BLA during performance of this spatial task. Consistent with lesion studies on conditioned place preference, this study suggests the BLA may give spatial information in the context in which learning occurs. [Supported by AG09299 and BNS 9120784]

578.14

The effect of α-THC on neuronal activity in the frontal cortical-basal ganglia system during a delayed match to sample task in rats. J.Y. Chang1, M.C. Lambeau2, K. Rall2 and D. J. Woodward, Department of Physiology and Pharmacology, Brennan Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Marijuana is currently one of the most prevalent substances of abuse. Its aversive effects include a disruption of motor and cognitive abilities. Cannabinoid receptors are densely concentrated in the frontal cortical and basal ganglia of rats, suggesting that behaviors that are dependent on the cortical-basal ganglia system should be sensitive to the effects of the cannabinoid. The present study employed ensemble recording to examine the effects of α-tetrahydrocannabinol (α-THC) on neuronal activity in medial frontal cortex (mPFC), striatum (STR) and substantia nigra pars reticulata (SNr) during performance of a delayed-match-to-sample testing. Animals were instructed to press a right or left sample lever and then execute nose-poke a delay period (~15 seconds). The first nose-poke after the delay period resulted in the presentation of both levers. Pressing the lever as in the sample phase resulted in a reward, whereas pressing the lever opposite to that presented in the sample phase resulted in a timeout period. Single and multi-unit activities were analyzed using peri-event histograms, joint peri-event time histograms and classification trees (CART). Alterations in spike activity associated with behavioral events in the sample, delay, and match phases were observed in mPFC, STR, and SNr. α-THC at doses of 0.5-1.0 mg/kg (i.p.) increased the occurrence of errors in the task in parallel with alterations in neuronal activity during the sample, delay and match phases. The data suggest that the frontal cortical-basal ganglia system is part of neuronal circuitry through which the cannabinoids may influence attentional and memory process. Supported by DA23181 W.W. server: htp://forms.bumc.edu.edu.

578.16

THE GABAB RECEPTOR, INPUT SELECTIVE PRESYNAPTIC INHIBITION AND THE REPRESENTATION OF FAMILIARITY
A.C. Tang1 and M.E. Hasselmo2 Department of Psychology and Program in Neuroscience, Harvard University, Cambridge MA 02138.

Recent physiological studies in brain slice preparations of the primate cortex (Tang and Hasselmo, Brain Res, 659, 75-81) demonstrated that the suppression of synaptic transmission (presynaptic inhibition) elicited by activation of GABAB receptors in the presynaptic terminal between pyramidal cells, but not for afferent input to the cortex. We have used computational models of cortical function to study how this differential influence on afferent versus intrinsic inputs to principal neurones plays a role in the judgement of the novelty or familiarity of stimuli. The model coupled pairs of excitatory, and 1 excitatory and 1 inhibitory cell connectivity among the excitatory neurones. We focused on the transient responses of individual neurones, showing that the perceived familiarity of a pattern of input can be represented by a quantity $S$ - the integral of the second inflection point of the neuronal response profile. Activity dependent presynaptic inhibition was crucial for an adequate representation of perceived familiarity in this model. Modeling allowed manipulation of network parameters corresponding to the presynaptic GABAB receptor density and the concentration of exogenous GABAB agonists (baclofen, used clinically to treat spasticity). Results show that either decreased GABAB receptor concentration on the presynaptic terminals, or increased exogenous GABAB agonist concentration, reduces perceived familiarity within the model. These results are consistent with the effect of chronic baclofen administration on rat behavior in an odor habituation task (Tang and Hasselmo, Behav. Brain Res, in press), the loss of recognition memory as a result of baclofen overdose, and the loss of GABAB receptors in Alzheimer's patients.

Supported by ONR N00014-93-1.356 and NIH R29 MH52732-01.

578.18

SPATIAL REPRESENTATIONS OF DORSAL CAUDAL NEURONS OF FREELY-BEHAVING RATS
J.S. J. Y. Mizumori1 and S.G. Cooper, Dept. Psychol., University of Utah, Salt Lake City, UT 84112

Experience-dependent visuospatial navigation requires not only continual update and modification of representations of our sensory surround as animals move through environments, but also continual refinement of movement patterns. With respect to the latter process, ventral striatal neurones show dynamic spatial and reward properties that may be used to direct navigational processes (Lacan et al., 1991). This study extends our stratial analysis by assessing the contribution of the caudate nucleus to control of navigational behavior.

Rats (8-12 mo old) performed a visual discrimination task with Food reward on a radial maze. 192 neurones were recorded in dorsal-rostral caudate nucleus of 7 rats. Consistent with Wiener (1993), a variety of spatial correlates were observed. Some spatial cells (>8) showed clear directionally-specific, spatially-localized firing. Another type of spatial coding was shown by 1 cell which exhibited mnemonic, directional heading properties: it fired when the animal's head was oriented in a preferred direction, but not regardless of the animal's location. Such directional firing could be maintained in the absence of visual cues or voluntary movement. 10 other cells also showed directionally-specific firing, but the firing occurred relative to the maze configuration. Additional spatial cells selectively fired when animals made right after left turns (n=12); 3 of these displayed turn correlates only when animals reversed direction. The caudate spatial messages are coded within multiple reference frames. Also, these data are consistent with primate studies which show that caudate neurones code context-dependently based on the guided learned behaviors. [Supported by AG09299 and BNS 9120784]

Thalamic and cortical amygdala-fugal pathways have been implicated in fear conditioning with an acrylic conditioned stimulus. Since infusion of NMDA-agonist APV in the amygdala disrupts fear conditioning, we studied the effect of APV infusion on neural transmission in the thalamo-amygdala and cortico-amygdala pathways, and on auditory evoked potentials in the lateral amygdala (LA). Urethane anesthetized rats were fitted with an auditory speaker for the delivery of a calibrated acoustic stimulus, and two stimulating electrodes: one in the medial division of the medial geniculate body and posterior intralaminar nucleus (MGIN/PIN) and the other in the TE5 region of the lateral amygdala. Average evoked potentials (EPs) elicited by these 3 stimuli (auditory-EPs, MGIN/PIN-EPs, and TE5-EPs) were measured in the lateral amygdala with a steel recording electrode equipped with an attached microcatheter (50 GΩ) loaded with APV (300 M). After stable baseline measurements were obtained, Sal of APV was infused, and all EP measurements were monitored at 15 min intervals. APV infusion reduced the slope of the auditory EP and MGIN/PIN-EP to <75% from baseline levels, and these responses showed recovery with time. APV infusion had no effect on TE5-EP, which remained stable throughout the measurement period. These results indicate that auditory-evoked activity in LA and transmission through thalamo-lateral amygdala synapses are largely dependent upon NMDA-receptor function. In contrast, transmission through cortico-amygdala synapses is not dependent upon NMDA receptor function. Supported by MH38774, MH48516, MH00956, MH10919.

PREDICTION OF LEARNED BEHAVIOR FROM ELECTROPHYSIOLOGY: DIMINISHED CONTRIBUTION OF LIMBIC THALAMUS TO AVOIDANCE RESPONDING AFTER EXTENSIVE OVERTRAINING IN RABBITS. M. Gabrieli, M. Hart, A. Poremba, Dept. of Psych. and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

Neurons in the limbic (anterior and medial dorsal) thalamic nuclei exhibit training-induced multi-unit activity (TIA) in rabbits learning to avoid a foot-shock by stepping into an activity wheel upon hearing a shock-predictive tone (CS+, 1 or 8 kHz) and to ignore a different tone (CS−, 1 or 8 kHz) that is predictive of shock. The TIA, increased tone-related discharges (excitation), which are greater in response to the CS+ than to the CS− (discrimination), reaches maximal levels in the limbic nuclei as behavioral learning reaches asymptote (see Gabrieli, M., in Neurobiology of Cingulate Cortex and Limbic Thalamus, 1993, Birkhauser, Toronto, 478-525). Bilateral electrolytic limbic thalamic lesions block learning (op. cit., 1993). The fact that TIA amplitude declines gradually during post-critical training (overtraining) suggested that the contribution of limbic thalamus to the learned behavior may diminish during overtraining. Here rabbits (n=5) given training to behavioral asymptote followed by limbic thalamic lesions showed significantly improved post-learning retention, relative to sham lesion controls (n=4), as measured by avoidance response performance during extinction testing (p<.04) and re-acquisition to criterion (p<.04). Ample retention was exhibited by other rabbits (n=5) given lesions after receiving training to criterion plus ten days of overtraining. This performance did not differ from that of similarly trained sham lesion controls (n=5). Thus, the limbic thalamic nuclei are involved in original learning and maintained performance of discriminative avoidance behavior, but are not critical for the behavior in highly overtrained rabbits. (Supported by NINDS grant NS26736 to MG).

MUTUAL INHIBITION OF NEURAL PATHWAY SYSTEMS DURING FORWARD AND BACKWARD CONDITIONING SUGGESTS SYMMETRICAL ATTENUATING MECHANISM DURING ASSOCIATIVE LEARNING. T. Kermerov*, T. Tansurinta and I. Arstikaitis, Dept. of Psychol., Univ. of Jyvaskyla, P.O. Box 35, 40351 Jyvaskyla, Finland.

Forward and backward conditioning procedures were used for an evaluation of the order effect of the conditioned (CS) and unconditioned stimulus (US) presentations. Six cats were first classically conditioned using tone-CS (1500 ms) delay paradigm in which a rewarding electrical stimulation train (500 ms) of the lateral hypothalamus served as the US. Both behavioral (head movements) and evoked neural responses were recorded in hippocampal areas (CA1, dentate fascia and subiculum) in freely moving cats. The result showed that during the forward pairing both the head movements and unconditioned evoked responses were significantly attenuated compared to the US-alone presentations.

Correspondingly, the responses to the CS (short latency, alpha responses) were significantly attenuated during the backward sessions compared to the CS-alone presentations. These findings suggest that the preceding stimulus leaves a temporal trace the effect of which temporally overlaps the subsequent stimulus. An assumption of the local postsynaptic interaction might explain the mutual inhibition effect of the converging CS and US pathways found in the present study. This conclusion is also supported by an observation of the specific modifying effect the US pathway on the CS pathway and vice versa.

THE AMYGDALA IS NECESSARY FOR THE INITIAL ACQUISITION BUT NOT FOR MAINTENANCE OF DISCRIMINATIVE AVOIDANCE BEHAVIOR IN RABBITS. A. Poremba* and M. Gabrieli, Dept. of Psych. and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

Bilaterial electrolytic amygdala lesions block acquisition of discriminative avoidance conditioning wherein rabbits learn to step in a large activity wheel upon hearing a footshock-predictive tone conditional stimulus (CS+, 1 or 8 kHz) and to ignore a different tone (CS−, 1 or 8 kHz), not predictive of shock. (Poremba and Gabrieli, Soc. Neurosci. Abst., 17:325, 1991). Here we asked whether the circuit necessary for the initial acquisition of the avoidance behavior is also involved in maintaining performance of the well-learned behavior. Rabbits (n=10) given bilateral intra-amygdaloid microinjections (0.5,8) of the GABA agonist, muscimol (1, mol), showed no signs of learning, either in terms of discrimination between CS+ and CS− or in terms of response incidence relative to a preliminary training session in which the CSs and the footshock were explicitly unpaired (p<.02). Rabbits given saline (vehicle) microinjections did develop discrimination (p<.02). Next, all rabbits (n=11) were trained to asymptotic performance and received 3 additional training sessions (overtraining). After this training, muscimol microinjections then significantly reduced performance relative to saline microinjections (p<.05). Finally, the rabbits received an additional seven days of training (n=4) or seven days of rest (n=5) followed by a muscimol and a saline session. In contrast to the findings after three days of overtraining, no significant reduction of avoidance responding was found. These results suggest that the amygdala is necessary for the initial acquisition of the discriminative avoidance behavior. Merely the passage of time after overtraining renders the amygdala unnecessary for maintenance of the avoidance behavior. (Support: NINDS grant NS26736 to MG).

In Alzheimer’s disease (AD) some of the earliest neurodegenerative changes include synaptic loss in the molecular layer of the dentate gyrus, as measured by synaptophysin immunoreactivity. The entorhinal cortex (EC) and basal forebrain (BF) are also affected, and reductions in forebrain choline acetyltransferase (ChAT; Perry (1986) Brain Pathol. 20; 42; 63). We and others have found similar changes in rats following electrolytic or tetrodotoxin (TTX) lesions of the EC or BF, and neurochemical lesions of the basal forebrain (BF). In the present series of studies we have characterised 3 groups of male, Lister hooded rats (young (Y) 3 months; middle aged (MA) 15 months; aged (A) 32 months) for behavioral, neurochemical and morphological differences to compare with those produced by BF or EC lesions.

Water maze testing revealed clear age-associated deficits in acquisition to find a submerged platform, which could be dissociated from changes in motivation/nosensor function. A subpopulation of A rats showed particularly poor acquisition e.g. day 4: path length Y=182+44cm, A unpaired (A) =311±45cm. A impaired (A) =531±14cm, p<0.01 vs. Y and A1 groups. Unlike EC lesioned rats, A rats (including A) showed no significant change in SI in the molecular region of dentate gyrus compared with control rats. Furthermore reductions in ChAT activity were much smaller than those produced by BF lesions, and were more prominent in the stratum, rather than hippocampus/torx. Thus in the present studies we have failed to find a simple correlation between age-associated changes in cognitive performance with such changes, perhaps indicating that multiple factors are likely to contribute.

579.5 THE INVOLVEMENT OF NUCLEUS ACCUMBENS IN LATENT INHIBITION. I. Weiner,1 G. Glaub,2 J. Fedorov,1,2 Dept Psychology, Tel-Aviv University, Tel-Aviv, Israel,1; Inst. Toxicol., Inst. Toxicol., Schorenstraat 16, Schwerzenbach 8603, Switzerland. (ENA )

When an organism receives repeated presentation of a stimulus without any other consequences, it subsequently disregards this stimulus when it is followed by significant consequences, e.g., reinforcement. This is reflected in slower acquisition as compared to the first novel stimulus and constitutes the phenomenon of latent inhibition (LI). LI is absent in rats and humans receiving the psychotomimetic, amphetamine, as well as in acute schizophrenic patients. Neuronal treatment restores LI in amphetamine-treated rats and schizophrenics. These results have led to the proposition that LI disruption models a cardinal cognitive deficit in schizophrenia, namely, an inability to ignore irrelevant stimuli. This proposal is strengthened by the fact that the neural substrates of LI parallel those implicated in the pathophysiology of schizophrenia, namely, the mesolimbic dopaminergic system. The implication of dopaminergic neurons in the control of mesolimbic dopamine transmission is further substantiated by the fact that the administration of a DA blocker, haloperidol, restored LI in lesioned animals. Thus, the capacity to ignore irrelevant stimuli critically depends on the integrity of the shell subterritory of the NAC (and therefore probably on the inputs received from this region in mesolimbic structures), and an impairment of such capacity is subserved by increased mesolimbic DA transmission. In addition, these results provide the first behavioral demonstration for the functional specialization of the shell and core subterritories of the NAC.

579.6 DIFFERENTIAL LEARNING-RELATED ACTIVITY OF HIPPOCAMPAL SINGLE UNITS DURING CLASSICAL CONDITIONING IN THE RABBIT. R.L. Rogers, M.A. Sanger and S.B. Berry. Center for Neuroscience Research and Department of Psychology, Miami University, Oxford, OH 45056.

While it is generally accepted that hippocampal cells discharge activity during behavioral classical conditioning, unit behavior analysis from several laboratories have indicated a surprising heterogeneity of responses among similar cell types both within and across hippocampal subfields (e.g., Kehoe et al. 1979; Wes et al. 1993). However, much of the research has been done in a single averse paradigm (rabbit NBD conditioning). To expand these findings, we recently showed that multiple unit activity recorded from CA1 corresponded to the behavioral response in the appetitive conditioned jaw movement paradigm (CIM. Oliver, Swan & Berry 1991). Given the variety of potential single unit response profiles (as in NBD conditioning), we asked to what extent single unit variables within hippocampal cell types during CIM.

New Zealand White rabbits were anesthetized (ketamine 50mg/kg and xylazine 10mg/kg) and unilaterally implanted with a stainless steel microelectrode. A cupped metal cylinder was cemented into place over the contralateral hemisphere that would later hold a micro-unit electrode unit and deprived of the right hemisphere. One month after surgery, the rabbits were trained for two days (34 trials per day) in CIM prior to attachment of the microdrive on the third day. Hippocampal unit responses and field potentials were recorded from both hemispheres. Some rabbits were trained in NBD/CS concurrent discrimination. Our results show that cells within the same electrophysiological classification (e.g., spike width 65±6 ms) respond differently during CIM, some showing diffuse increases in firing rate to the tone, others showing periodicities similar to the rhythmic jaw movement response. Some units are from animals that were trained in a concurrent NM and CIM discrimination task to assess whether the same cell responds differently during performance of the two distinct behavioral responses.

579.7 HIPPOCAMPAL CA1 SINGLE NEURON ACTIVITY DURING TRANCE EYEBLINK CONDITIONING. C. Neafy,1 M.R. Kempefer,2 and J.F. Dutschke. Dept. of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL 60611.

Multichannel recordings were used to monitor the activity of several hippocampal CA1 pyramidal cells simultaneously during trace eyelid conditioning in the rabbit (100 ms tone, 500ms trace, 150 ms airpuff). Control rabbits were presented with unpaired tones and airpuffs. The Datawave software package was used to separate the activity of single neuron activity by paired pulse electrode. Pyramidal cells were separated from interneurons according to spike width, spike rate and the presence of a consistent tone-evoked response. Activity during conditioning was monitored by recording contingent responses to a novel stimulus and constitutes the phenomenon of latent inhibition (LI). LI is absent in rats and humans receiving the psychotomimetic, amphetamine, as well as in acute schizophrenic patients. Neuronal treatment restores LI in amphetamine-treated rats and schizophrenics. These results have led to the proposition that LI disruption models a cardinal cognitive deficit in schizophrenia, namely, an inability to ignore irrelevant stimuli. This proposal is strengthened by the fact that the neural substrates of LI parallel those implicated in the pathophysiology of schizophrenia, namely, the mesolimbic dopaminergic system. The implication of dopaminergic neurons in the control of mesolimbic dopamine transmission is further substantiated by the fact that the administration of a DA blocker, haloperidol, restored LI in lesioned animals. Thus, the capacity to ignore irrelevant stimuli critically depends on the integrity of the shell subterritory of the NAC (and therefore probably on the inputs received from this region in mesolimbic structures), and an impairment of such capacity is subserved by increased mesolimbic DA transmission. In addition, these results provide the first behavioral demonstration for the functional specialization of the shell and core subterritories of the NAC.

579.8 INCREASED SYNAPIC RESPONSIVENESS BETWEEN CA3 AND CA1 AFTER TRACE EYEBLINK CONDITIONING RECORDED IN VITRO. J.M. Power,1 L.T. Thompson, J.R. Moyer,2 and J.F. Dutschke. CM Biology, Northwestern University Medical School, Chicago, IL 60611.

Thirty-four young (2 month) female New Zealand rabbits received either trace conditioning (80 Hz; daily 1 h 100 ms tone CS; 500 ms trace ISI; 150 ms airpuff, pseudoconditioning, or were naive. Conditioned animals were trained daily until reaching a criterion (CS duration and airpuff 1 s; three trials per day) of 10 s of conditioned response (CR) before the CR was selected 1 h 24 h following the final training session. Field potentials were evoked by stimulation of the Schaffer collaterals, using a stainless steel bipolar electrode. Positive and negative potentials were recorded in CA1, 750 μm from the stimulating electrode. An input-output function for each slice was generated by using a range of stimulus durations (20 - 600 μs) encompassing subthreshold to maximal population spike responses. The maximal population spike amplitude was 39% greater in slices from conditioned animals sacrificed 1 h after acquisition and 24% greater in slices from conditioned animals prepared 24 h after conditioning. Additionally, the EPSP slope was found to be greater in slices prepared from conditioned animals 1 h post acquisition. Field potentials from naive and pseudoconditioned neurons were indistinguishable. Recording and data analysis were completed within 3 h of the trace conditioning. Field potentials from conditioned and pseudcondioned neurons were indistinguishable. Further analysis and experimentation are being done to determine whether the enhanced population spike amplitudes result from altered synaptic efficacy, increased postsynaptic excitability, or both.

Supported by NIH R37 MH43739 and AG08796.
TS5.9

CLASSICAL CONDITIONING USING NUCLEUS BASALIS STIMULATION PRODUCES ASSOCIATIVE CS-SPECIFIC DESYNCHRONIZATION IN THE AUDITORY CORTEX EEG. J. Haskin, S. Bjordahl, and N. Weisberger, Center for the Neurobiology of Learning and Memory, and Department of Psychology, University of California, Irvine, CA 92717

Cholinergic system activity plays a critical role in learning and memory, cortical plasticity, and cognitive plasticity (of Weisberger & Haskin, 1993 Current Opinion Neurobio). Classical conditioning using an auditory conditioned stimulus (CS) produces specific long lasting plasticity in primary auditory cortex (ACx) receptive fields (RFs), resulting in changing RFs to or around the stimulus frequency (Haskin and Weisberger, 1990 Br J Psychol). We hypothesized that this plasticity requires the convergent activation of both cholinergic and cholinergic modulatory inputs to the ACx (Weisberger et al, 1990 CNS). As an initial test of this model, we classically conditioned urethane anesthetized adult male rats, substituting electrical stimulation of the nose for footshock, as the unconditioned stimulus. Neuronal RFs and cortical EEG were simultaneously obtained from ACx ipsilateral to the NB stimulation and contralateral to the CS speaker. Here we report the effect of classical conditioning with NB stimulation on ACx EEG.

NB stimulation alone (500ms, 200Hz, 100-300pA) produced EEG desynchronization (14/14 subjects, p<0.003) that could be blocked by atropine (5S, p<0.05). Auditory tones alone did not (5/6, p>0.43). During RF characterization following tone/NB conditioning (40 trials), the CS frequency (p>0.05), but not non-CS frequencies (p<0.01) produced EEG desynchronization. Sensitization controls did not develop CS-induced EEG desynchronization (p>0.26), indicating that this effect is associative. This CS-specific EEG desynchronization could be detected 30 minutes post training, the longest interval tested.

These results demonstrate that convergent activation of acoustic and cholinergic inputs to ACx can support plasticity that might play a role for ACx RF plasticity during learning. Supported by NINDC RO02346 (NIMH).  

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TS5.10

MULTIPLE-UNIT RECORDING OF THE INTERPOSITUS NUCLEUS DURING CLASSICAL CONDITIONING OF THE RABBIT EYELID RESPONSE TO INTERMODAL (LIGHT/DARK)-SIMULATED L. I. Basso and J. A. Labor, Emory University, Atlanta, GA 30322

The interpositus nucleus (INP) is believed to be essential for the acquisition of a classically conditioned eyelid response (CR) following pairings of either light or tone conditioned stimuli (CS) with an air puff. Previous single-unit and multiple-unit recordings from the INP have revealed neuronal activity that precedes and models the CR. Allen and Steinmetz, 1994 found sites in the cerebellar cortex that modeled CRs to two different tone frequencies, some of these toning a long interstimulus interval (SI), the other signalling a short ISI. The present study sought to determine whether recording sites in the INP could be found that would model CRs to stimuli from two distinct sensory modalities.

Rabbits were surgically implanted with multiple-unit electrodes in both the left and right INP and trained on each side with both tone and light CS until reaching a criterion of 70% or more CRs for two consecutive days. In each session the rabbit received 120 CS-US pairings of a 350 ms CS that co-terminated with a 100 ms air puff in a standard delay conditioning paradigm. Activity was recorded from the INP throughout training.

Preliminary findings indicate tone-related CR activity at sites within the INP, but no recording sites have modeled CRs to both tone and light CSs. Examination of the histological data reveals that thus far the sites sampled have been from lateral sections of the INP. These results would suggest that separate pathways exist through the INP for encoding the light versus tone CS-US associations. We are currently recording from other deep nuclear areas in an attempt to localize sites where light CS-related activity can be found. Supported by NIMH grant # MH51178.

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TS5.11

IMMUNOHISTOCHEMICAL EXPRESSION OF THE c-FOS PROTEIN IN THE SPINAL TRIGEMINAL NUCLEUS FOLLOWING PRESENTATION OF CORNEAL AIRPUFFS. M. B. Schnell, S. K. McCauley, T. J. WEBBER, E. J. Green, Janice M. Hitchcock, and N. Schneiderman, Behavioral Neurogenetics Program, Dept. of Psychology, University of Miami, Coral Gables, FL 33124

Previous work has shown that pairing a tone-conditioned stimulus (CS) with corneal-airpuff-uncorrelated stimulus (US) produces reliable heat rate (HR) conditioning. A major goal in the neurobiological study of this HR conditioning paradigm is to localize potential sites of plasticity in the中枢 nervous system (CNS) where CS and US information converge. The circuits involved in the processing of the tone-CS has been examined in some detail, however, less is known about the CS-US-US information reaches cortical sites of plasticity in the CNS. Therefore, the present study examined the expression of the c-Fos protein in the natal's CNS to determine which areas are activated by the paired tone and corneal airpuff US. In this study restrained awake rabbits received 100 presentations of a corneal airpuff. Brains were then removed and coronal sections were processed immunohistochemically for the c-Fos protein. In animals that received the airpuff, the ventral portion of the ipsilateral spinal trigeminal subnucleus caudalis (SVC) and interpontics (SIV), and the dorsal oral nucleus exhibited a greater number of c-Fos labeled cells per coronal section compared to control animals. Another group of animals was given microinjections of WGA-HRP in the medial nucleus of the medial geniculate (MGN) to determine if this critical area of the HR conditioning circuitry receives projections from SVC and SIV. These injections produced retrograde labeling in the same areas of SVC and SIV activated by the airpuff. Thus, a corneal US activates neurons in SVC and SIV which can then activate neurons in MGN. This provides additional evidence that CS and US information converge in MGN, an area believed to be a site of plasticity in the HR conditioning pathway. Supported by NSF IB0022194 and NIH HD07426.
759.15
RESPONSE CHARACTERISTICS OF VENTRAL SEGMENTAL NEURONS IN THE AMYGDALA AND HIPPOCAMPUS DURING STRESS. F.-A. Quirion and S. Insp, Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405.

A great deal of research has indicated that the mesocorticolimbic dopamine (DA) system plays an important role in reward and aversive processes. Nevertheless, there has been a paucity of research describing in detail the response properties and characteristics of single DA neurons within the ventral tegmental area (VTA) of awake animals, particularly in response to stimuli predictive of aversive events. The present experiment is one of a series designed to investigate the response characteristics of VTA neurons to a variety of novel auditory stimuli (e.g., pure tones, white noise) and to auditory stimuli which predict aversive events in the awake rabbit. Single-unit recordings within the VTA were isolated and identified according to established criteria: spontaneous rate of 0.8-10 Hz and >2.0msec spiketrain stimulus presentations. The spontaneous activity of these neurons was characterized by single spikes interspersed with bursts of 2-6 spikes with a mean burst interspike frequency of approximately 60/sec. In response to auditory stimuli, three distinct response patterns were observed among different VTA neurons. The first was an increase in activity within 100msec of stimulus onset which was often characterized by a spike burst. The second response pattern was characterized by a marked inhibition to stimulus onset which outlasted the duration of the stimulus. The third pattern was characterized by a lack of response to auditory stimuli. Additional, preliminary data suggest that these three response patterns also occur in response to Pavlovian avermically-conditioned auditory stimuli. These results suggest that DA neurons within the VTA of the rabbit are responsive to novel auditory stimuli as well as to auditory stimuli predictive of aversive events.

759.16
ANALYSIS OF GENE EXPRESSION ASSOCIATED WITH MEMORY CONSOLIDATION IN RATS USING THE DIFFERENTIAL DISPLAY METHODOLOGY. M.-L. Huang and E. H. Y. Lee. Graduate Institute of Life Sciences, National Defense Medical Center 1 and Institute of Biomedical Sciences, Academia Sinica 2, Taipei, Taiwan, R.O.C.

Involvement of gene expression associated with memory formation has been examined in learning paradigms for some invertebrates and vertebrates. However, little is known in the paradigm of inhibitory avoidance learning in rats. In this study, a PCR-related protocol has been used to analyze differential gene expressions related to memory consolidation. Total hippocampal RNAs isolated from animals showing poor and good retention scores were used for comparison. Three 3-primer (TaqI, TcrI, and TaqII) and four 3-4 arbitrary 10-mers were used. Each primer pair generated an average of 50-70 cDNA bands and most of the bands between individuals are identical. Several bands were found to be differentially expressed between poor and good memory rats. In one particular case, only a 3'-end primer (TaqAG) was used for PCR amplification. Two cDNA bands, designated as upper and lower band, showed differential expression between individuals of poor and good memory. These two bands were separately recovered from the gel, cloned into a TA vector, and sequenced. Both of them showed 90% homology with the 3'-end cDNA sequence of the glutamatergic acid protein (GFAP). For further examination, 13 poor and 13 good memory individuals were compared. In the poor memory group, the lower cDNA band was found in 12 individuals. However, only 5 individuals in the good memory group showed this band. The try to physiologically transcribed sequences exist in the population and one of them may be associated with memory consolidation. cDNA clones of these two different transcripts will be isolated from the individual hippocampal cDNA library and the differences will be characterized.

759.17
ENHANCED HIPPOCAMPAL CRF GENE EXPRESSION ASSOCIATED WITH MEMORY CONSOLIDATION IN RATS. E.-H. Y. Lee1, A.M. Huang and K.S. Tsai2. Biomed. Sci., Academia Sinica 1, Taipei 115, Taiwan, R.O.C.

Corticortin-releasing factor (CRF) was found to produce various behavioral changes other than its neuroendocrine function. We have previously found that intra-hippocampal injections of CRF significantly improved memory retention of an inhibitory avoidance learning task in rats and CRF antagonist prevents this effect. Other laboratories have also found that i.v. injections of CRF enhance acquisition/learning in inhibitory avoidance learning paradigms in rats and mice. CRF gene expression was demonstrated in various brain regions including the hippocampus. The present study used the quantitative RT-PCR method to examine CRF gene expression changes during memory consolidation. One-way inhibitory avoidance learning paradigm was adopted. Results indicated that during the early phase of memory consolidation, which is 1 hr and 3 hr after the training procedure, there was a marked increase of CRF mRNA level in the hippocampus in animals showing good memory when compared with those showing poor memory. These results suggest that CRF gene expression is enhanced during the memory consolidation process.

759.19
BEHAVIORAL STRESS IMPAIRS HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) THROUGH A BLOCKAGE OF N-METHYLDOPAMINE D-ASPARTATE (NMDA) RECEPTORS. J. J. Kim1, M. R. Roy1 and R. F. Thompson2. 1Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90039-2520 and 2Psychology Dept., Loyola Marymount Univ., Los Angeles, CA 90045-2669.

Behavioral stress is known to impair various learning and memory tasks, and also to impair the process of LTP. Our previous work has shown that exposure to a novel environment induces subsequent LTP development by elevating the basal synaptic transmission level. Since the induction of LTP in the hippocampus (e.g., area CA1) is known to require activation of NMDA receptors, we tested whether NMDA antagonists can block the stress effect on subsequent LTP. Adult Long-Evans male rats (200-350 g) were administered intraperitoneally (i.p.) with either the NMDA receptor antagonist which crosses blood brain barrier, or saline 2 hours prior to stress. The stress consisted of 60 tailstrokes (15cm, 1.4, 30-90/ml apart) while restrained. Following stress, hippocampal slices were prepared in a standard manner. When initial slopes of field-EPSPs were examined in stratum radiatum in CA1 following stimulation of Schaffer collateral/commisural fibers, we found that slices from saline-stressed animals exhibited significantly impaired LTP (T0/T0+4.9%, mean ± SEM) after tetanus in comparison to slices from saline-control (141.6±4.9%), CGP55851-control (140.4± 8.0%) and CGP53925-stressed (151.9±9.8%) animals. The CGP53925 effect appears to be dependent on neuron damage since animals injected with the anxiolytic drug diazepam (5 mg/kg ip) and then stressed were still impaired in LTP. Thus, our results suggest that the process by which stress impairs LTP appears to be mediated through NMDA receptor activation. Supported by grants from NRSA 1F32MH105121-01 BNR to JJK, LMU to MRB, and NSF IBN9215069, NIA AF05142 and Sankey to RFT.

759.18
ALTERATION IN THE ACTIVITY OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II ASSOCIATED WITH SPATIAL LEARNING IN RATS. S.F. Tan1, R.K. Chong2, S. Chua3, C.K. Leong2 and S.M. Lasteyrie. 1North Carolina, Chapel Hill, NC 27599, USA; 2RTP, NC 27711, and 3U.C. College, Peoria, IL 61656.

Childhood lead (Pb) exposure has been associated with impaired cognitive function. Previously, we employed the hippocampal long-term potentiation (LTP) memory model system and demonstrated an increase in the threshold for LTD potentiation in the untrained group. The present study examined the induction and time course of decay of LTP in the dentate gyrus of the conscious rat chronically exposed to Pb. Prior to testing, male rats were exposed 0.2% Pb-acetate in the drinking water and male offspring were maintained on this solution. As adults, animals were prepared with chronic stimulating/recording electrodes in the hippocampus. Following recovery from surgery and the establishment of stable field potentials evoked by single pulse stimulation (0.1 ms) of the perforant path, a pretrain input/output (I/O) function was collected. LTP was then induced by delivering 10 train pairs (4 pulses/pair at 400 Hz), 200 ms apart, with an intertrain interval of 10 s at 6 stimulus intensities (200-1000uA). Three additional I/O functions, 1 hr, 1 day, and 1 week following tetanization were obtained to monitor induction and decay of LTP. Comparison of pre- and post- train I/O functions revealed a significant enhancement of PS amplitude in the control group, but no difference from pretrain levels in the Pb-exposed group at any timepoint. These data suggest that the detrimental effects of developmental Pb exposure on cognitive function may be due to its interference with the synaptic processes underlying LTP. (Supported by NIH ES06523 and US EPA)

759.20
PRENATALLY-INITIATED LEAD (Pb) EXPOSURE IMPAIRS HIPPOCAMPAL LTP IN THE AWAKE RAT. M.E. Gilbert1, D.C. M. Mack2, and S.M. Lasteyrie. 1North Carolina, Chapel Hill, NC 27599, USA; 2RTP, NC 27711, and U.C. College, Peoria, IL 61656.

Childhood lead (Pb) exposure has been associated with impaired cognitive function. Previously, we employed the hippocampal long-term potentiation (LTP) memory model system and demonstrated an increase in the threshold for LTD potentiation in the untrained group. The present study examined the induction and time course of decay of LTP in the dentate gyrus of the conscious rat chronically exposed to Pb. Prior to testing, male rats were exposed 0.2% Pb-acetate in the drinking water and the animals were maintained on this solution. As adults, animals were prepared with chronic stimulating/recording electrodes in the hippocampus. Following recovery from surgery and the establishment of stable field potentials evoked by single pulse stimulation (0.1 ms) of the perforant path, a pretrain input/output (I/O) function was collected. LTP was then induced by delivering 10 train pairs (4 pulses/pair at 400 Hz), 200 ms apart, with an intertrain interval of 10 s at 6 stimulus intensities (200-1000uA). Three additional I/O functions, 1 hr, 1 day, and 1 week following tetanization were obtained to monitor induction and decay of LTP. Comparison of pre- and post- train I/O functions revealed a significant enhancement of PS amplitude in the control group, but no difference from pretrain levels in the Pb-exposed group at any timepoint. These data suggest that the detrimental effects of developmental Pb exposure on cognitive function may be due to its interference with the synaptic processes underlying LTP. (Supported by NIH ES06523 and US EPA)
T90.21
Hippocampal LTP and Spatial Learning are Attenuated by GABA_B Receptor Blockade in the Rat
E.H. Brunstroem, P.D. Lewis, D.D. Moff, D.V. Lewis, W.A. Wilson* and H.S. Swaabwilder. Departments of Medicine, Pediatrics, Psychiatry, and Pharmacology, Duke University Medical Center and Neurosciences Research Laboratory, V.A. Medical Center, Durham, N.C., 27705.

This series of experiments assessed the role of GABA_B receptors in the induction of long-term potentiation (LTP), and spatial learning and memory using three different tasks. In urethane-anesthetized rats, theta burst stimulus trains were applied to the angular bundle and recorded in the dentate gyrus. Control LTP was attenuated from a mean of 145% to 57% in CFP 46381 treated rats (t(10)=2.530, p=0.028, n=14).

We assessed the performance of rats on several spatial learning and memory tasks in rats. We found that the working memory performance of highly trained rats on the eight arm radial maze was unaffected by CFP 46381. There was also no effect of GABA_B receptor blockade on the eight arm maze using a repeated acquisition paradigm. However, when we tested spatial learning in naïve rats using the Morris water maze, we found that CFP 46381 treated rats (n=12) took significantly longer (p<0.05) to learn the location of the escape platform than control (n=11). In addition, CFP 46381 treated rats travelled a greater distance over the acquisition and probe trials than did control rats. These data support our hypothesis that GABA_B receptor blockade suppresses hippocampal LTP in vivo and impairs spatial learning in a task where stress may be important.

Supported by a VA Merit Review grant to HSS and a Tobacco Research Council grant to EDL.

T60.1
THE CONTRIBUTION OF THE MEDIAL SEPTUM TO THE FUNCTIONS OF THE HIPPOCAMPAL SYSTEM IN RATS. P. Nunan.* Psychology Department, Santa Clara University, Santa Clara, CA 95050.

In people, consensus now favors the view that the septohippocampal system regulates declarative memories. However, in non-human animals do not have language ability, it has been difficult to identify the analogue of declarative memory in these species. We review experiments from our laboratory on the effects of septal lesions on the behavior of male Long-Evans hooded rats, and conclude that rats may 'decide' what they know in terms of their volitional behavior, and that the working memory for such voluntary goal directed behavior is disrupted by medial septal lesions. In one experiment (Nunan and Guaranta, 1990), medial septal lesions impaired a left-right operant delayed alternation task which appeared to be caused by a deficit in working memory. In a second experiment (Nunan and Klin, 1992) we found that medial septal lesions facilitated performance on a delayed but go/no-go stimulus working memory task, suggesting that the lesions do not produce a general working memory impairment. In a final experiment (Nunan, 1994) we found that medial septal lesions impaired performance on a delayed go/no-go response alternation task suggesting that the deficit is specific to response working memory, and that spatial task requirements are not necessary for the expression of such a deficit.

We suggest that the septohippocampal system functions as a response intention--response outcome working memory comparator and that while damage to the system impairs the working memory for voluntary behavior, it may also lead to a compensatory reliance on, and hence a potential enhancement of the functions of, eortocortical cue based memory systems.

T60.2

Intravenous injection of the immunoxtxin, 192-sap, that selectively destroys neurons expressing the low affinity neurotrophin receptor, p75NK, efficiently and selectively destroys the cholinergic neurons of the basal forebrain (CBF). In the present study, we sought to determine if there was a correlation between the degree of CBF neuron loss and alteration in passive avoidance behavior. Anesthetized, adult, male Sprague-Dawley rats were stereotactically injected with 4 mg of either 192-sap or OX7-sap, a control immunotoxin that recognizes the Thy 1 surface antigen and destroys cerebellar Purkinje neurons. 6-8 weeks later, immunoxtxin and naive control rats were tested on a step-through passive avoidance paradigm. After behavior testing, all rats were sacrificed and brain sections processed for histochemical demonstration of AChe and immunohistochemical demonstration of p75NK. The numbers of neurons in specific regions of the CBF were counted from the p75NK staining and the intensity of dorsalized neocortical staining for AChe were assessed using image analysis. The magnitude of cell loss was similar (67-70%) for the entire CBF, the Nbm and septum/DHB. The severity of passive avoidance impairment was significantly correlated to cell loss in the entire CBF (r=0.748, 23 df, p=0.001) and the Nbm (r=-0.778, 23 df, p=0.001) but not the septum/DHB (r=0.419, 23 df, p=0.05). Behavioral impairment also correlated significantly to loss of cortical AChe staining intensity (r=-0.796, 15 df, p<0.001). These findings show that loss of CBF, but not of septum/DHB, cholinergic neurons is proportional to impairment in passive avoidance behavior suggesting a role for Nhnoocortex cholinergic innervation in this type of learning. (Supported by the Department of Veterans Affairs.)

T60.3
INTRAEPITHELIAL INJECTION OF 192 IgG SAPORIN, AN IMMUNOTOXIN, REDUCES LATE INHIBITION IN A CONDITIONED TASTE AVERSION PARADIGM. K.D. Dougherty*, A. Dull, D.R. Wala, L.L. Rutgers University, New Brunswick, NJ, 08803.

When animals are pre-exposed to a CS prior to its pairing with a US, the acquisition of the conditioned response is impaired. This phenomenon is called late inhibition (LI) and is often used as a measure of selective attention. The role of cholinergic mechanisms in selective attention was assessed using a conditioned taste aversion/ LI paradigm. 192 IgG saporin (SAP) (375 or 100 ng/g,0.04 ml) or artificial cerbrospinal fluid (aCSF) (0.45ml) was injected into the medial septal area of male Sprague-Dawley rats. aCSF subjects given 4 pre-exposures to a novel saccharin (0.25% solution prior to its pairing with lithium chloride (0.05%)) later displayed robust LI during a two bottle choice test. In contrast, subjects treated with either 100 or 375 ng SAP displayed significantly attenuated LI. Nonpre-exposed SAP rats displayed averted saccharin to extinction ratios of comparable to those of controls. Both 100 and 375 ng SAP significantly high affinity choline uptake (HACU) in the hippocampus and cingulate cortex. Only the 375 ng dose caused significant HACU decreases in entorhinal cortex. HACU was not affected in striatum. HACU analyses performed in a subset of subjects revealed no effect of SAP on regional concentrations of norepinephrine, serotonin, dopamine or their metabolites in hippocampus. The results indicate that there may be considerable overlap between the cholinergic systems that mediate working memory and those that are critical to selective attention.

T60.4
Effects of 192 IgG Saporin Induced Lesions of the Medial Septum on Two Allocentric Spatial Memory Tasks. L.E. Janie*, Z. Filip, and D.G. Stain Brain Research Lab, Inst of Animal Behav, Dept of Psychology, Rutgers University, Newark, New Jersey, 07102.

Previous studies have suggested that the hippocampal system is important for processing allocentric spatial information and that damage to the medial septum (MS) impairs an animal from using allocentric information to solve spatial tasks. Because the cholinergic input to the hippocampus via the MS is necessary to maintain normal electrical activity and normal physiological functions of the hippocampus, we sought to determine whether hippocampal allocentric spatial ability was dependent upon this input. Twenty male rats were initially trained on a win-shift radial arm maze (RAM) task and then given either sham or cholinergic-induced (192 saporin) lesions of the MS. Rats were then tested postoperatively on the RAM to assess their allocentric spatial ability. The results indicated that while rats with cholinergic MS lesions were significantly impaired in resolving the task, they did not exhibit deficits in allocentric spatial ability. In addition to RAM testing, rats were also tested on a modified water maze task designed specifically to test allocentric spatial ability. In this task, rats with cholinergic MS lesions performed as well as sham rats. Taken together these results suggest that the cholinergic projection from the MS is not critical for normal allocentric spatial ability and that such ability can be maintained by the remaining septohippocampal input. Supported by GenRe Corp. and Sigma Xi student research grant.
106.5 LATERAL ENTORHINAL CORTEX DAMAGE: ASSOCIATED EFFECTS OF "ODOR-PLACE" LEARNING IN RATS. T. Ono*, K.M. Schiller, O. Coenen, & C. Ding. Program in Biopsychology and Behavioral Neuroscience, Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

Neuropsychological, anatomical, and electrophysiological data indicate that the lateral entorhinal cortex (LEC) is an important area for the convergence of olfactory information and hippocampal processing during learning. In an attempt to explore the potential functional role of the LEC in the acquisition of the relations between olfactory and spatial stimuli, we have recently developed an "odor-place" task in which rats are required to learn to go to one location in the presence of a particular odor and to a second location in the presence of a different odor in order to obtain water reinforcers. Thus, accurate performance in this task requires learning the relationship between a specific odorant and a discrete spatial location. Bilateral aspiration of the LEC resulted in a marked deficit in acquisition of this task relative to sham-operated subjects, control experiments indicate that this impairment is not due to a sensory deficit. These data are consistent with the notion that the LEC participates critically in the acquisition of associations between multiple, discrete stimuli.


Work with both monkeys and rats has shown that damage limited to the rhinal cortical region (entorhinal cortex + perirhinal cortex) is sufficient to produce severe impairments in object-recognition memory (Murray et al., 1989; Marmus et al., 1994). In primates, this impairment is largely due to the perirhinal component of the lesion, as lesions of the perirhinal cortex (PrCs) produces deficits nearly as severe as those seen after complete rhinal cortex lesions, whereas ablation of the entorhinal cortex produces only a mild deficit (Marmus et al., 1993). To assess the contribution of the perirhinal cortex to object memory in rats, we compared the performance of animals with lesions limited to this area to that of animals with combined perirhinal and entorhinal cortex lesions on a rotation version of DiMeo's object-discrimination task, which resembles those used to study amnesia in humans and monkeys (1) object discrimination, (2) discrimination reversal, and (3) heteroconcurrent object discrimination learning.

Male Long-Evans rats received either bilateral lesions of the perirhinal cortex alone (Pr), perirhinal + rhinal cortex lesions (PrR), or sham surgery (Co). The Pr group was significantly impaired with respect to the Co group on postoperative DiMeo's test at delays of 60 sec (p<0.05) and impaired with respect to both control and perirhinal-lesioned rats at delays of 120 sec (p<0.01). Although the DMNs scores of the Pr group were lower than the controls at almost all delays, no statistical differences were found. On the simple object discrimination task, both the Pr and Rg groups were equally impaired relative to the control rats (p<0.02). There were no statistical differences, however, in the rate at which all three groups acquired the discrimination reversal. With respect to the concurrent object discrimination task, a significant impairment was noted when the PrR and Rg animals were combined into a single, uniform group (p<0.03). These results provide further evidence for the importance of the rhinal cortical area in object-memory processes in the rat.

106.9 PERIRHINAL CORTEX DAMAGE: EFFECTS ON ACQUISITION AND RETENTION OF OBJECT AND PLACE DISCRIMINATIONS IN RATS. R.S. Adol® D.G. Mcnab, and R.J. Sutherland. Dept. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

In order to examine the effects of perirhinal cortex lesions on object memory, 12 rats received bilaterally transected olfactory tracts, and 14 rats received bilateral lesions of the perirhinal cortex, and 6 rats received lesions of the entorhinal cortex. Retention tests were assessed by testing all rats on five object discriminations and the three pool tasks that they had learned previously. Anterograde effects were assessed by testing the rats two new object discriminations and one new pool task, and the old object discriminations, there were no significant differences in the groups in learning the discriminations nor in their accuracy in the first five trials of testing. For the new object discriminations, there were no significant differences between the groups in learning the new object discriminations. For the old pool tasks, there were no differences between groups in percent of time spent in the correct quadrant during probe trials or first-trial escape latency, although there was a trend for the perirhinal group to be impaired on these measures. These data suggest that perirhinal cortex plays a nonessential role in the acquisition and retention of object and place discriminations.

106.10 POSTTRAINING LESIONS OF PERIRHINAL CORTEX DECREASE PASSIVE AVOIDANCE OF PUNISHED DRINKING BUT NOT ITS REINSTATEMENT. G.D. Cooper*, and K.J. Mentis. Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.

Lesions of the rostral perirhinal cortex (rPrCs) of the rat disrupt fear-conditioned to discrete stimuli (Rosen et al., 1992; Rosen et al., 1993) and also conditioned stimuli (Corsoian and DeLoux, Soc. Neurosci. Abstr. 20-1007, 1994). This present study examined whether rPrCs would disrupt the context-dependent memories of previously-learned passive avoidance of (PD) male Long-Evans rats were water-deprived and trained to a criterion of 5 min avoidance of drinking by punishing drinking with 5-sec footshocks (FS) that increased in intensity over trials. They learned within 11-20 FS (Mdn = 16, M = 15.9, SD = 2.6). Five days later they received either control surgery or 1-mA/sec tonic anodal lesions of the rPrCs or the rostral central nucleus of the amygdala (aRca). Two weeks later latency to drink without further PD was assessed in the testing chamber. They were allowed to drink for 30 sec. Rats not returning to drink within 3 min were given another session the next day. All rats were given two additional, daily sessions of 1-min cumulative drinking and then tested for reacquisition of PD. When tested twice after surgery, the 8 control rats took at least 16 sec to drink (Max = 788, MSExp = 375.09). All 4 PD rats drank in less than 98 sec (Min = 34, MSExp = 6147). The aRca group also returned sooner than the control group (Range 50-220, MSExp = 142). Despite the apparent absence of acquisition of avoidance by controls, they did not reacquire PD with fewer FS trials (MSExp = 3.23±0.49) than the rPrCs group (MSExp = 3.67±0.24). The aRca group exhibited a major deficit in PD by requiring 32.6±3.6 FS for reacquisition vs. 5 min. In reacquisition, the rPrCs rats actively avoided the spout after the 1st FS (3 rats) or 2nd FS (the other 6) and they exhibited avoidance of the spout on that trial (6 rats) or after one more FS (the other 3). The lesions appeared to alter the "state" in which memory of the punishment contingency was being retrieved.
760.11
AMYGDALA GABA\(_\text{A}\) RECEPTORS MODULATE RETENTION OF CHANGES IN REWARD MAGNITUDE. 
LA Sheline* & J. J. McGaugh.
Center for the Neurobiology of Learning & Memory and Dept. of Psychology, UCLA, Los Angeles, CA 90095.
We have previously shown that post-training amygdala inactivation impairs memory for reward retention. In the present study we infused a GABA\(_\text{A}\) agonist (muscimol, MUS) or antagonist (bicuculline, BIC) into the amygdala immediately after a reward. Rats with bilateral amygdala cannulae were trained to run a straight alley for a large or small food reward. In Experiment 1 rats in the large-reward group were shifted to the small reward and an infusion of vehicle or MUS bilaterally into the amygdala immediately after. Shifted vehicle animals displayed an increase in runway latencies compared to unshifted controls. In contrast, shifted MUS animals displayed latencies comparable to unshifted animals by the second postshock day. These findings suggest that muscimol attenuated the memory of the reward and those that received bicuculline. In Experiment 2 rats that were shifted before except they received BIC immediately after reward shift. Shifted BIC animals displayed increased runway latencies compared to shifted vehicle animals by the second postshock day. These findings suggest that BIC enhanced memory of the reward shift. In Experiment 3, animals were trained as before except they first experienced a reward increase before receiving post-training injections of vehicle, MUS or BIC. On the next day the reward was reversed. Despite reward shift, shifted BIC animals persisted displaying low latencies for more trials than shifted MUS animals. These findings suggest that BIC enhanced memory for the reward increase. Further, the findings suggest that the amygdala and its GABAergic system is involved in memory consolidation for both positive and negative affective experiences.
Supported by APA fellowship 2-T32 MH18882 (JAS) and PHS MH51256 (NIMH and NIDA) (JLM).

760.12
BASOLATERAL AMYGDALA LESIONS BLOCK GLUCOCORTICOID-INDUCED MODULATION OF MEMORY FOR SPACIAL LEARNING. B. Benedetti*, C. Prilofsky, G. McGaugh, Center for the Neurobiology of Learning and Memory, and Dept. of Psychology, Univ. of California, Irvine CA 92717-3800.
This study examined the involvement of the amygdala in the effects of glucocorticoids on the formation of memory for spatial learning in a water maze. Male Sprague-Dawley rats were given five training trials of spatial orientation learning in a water maze, and retention was tested 48 h later. Removal of the adrenal glands (adrenalectomy; ADX) 4-5 days prior to training significantly impaired memory for this task, and immediate posttraining systemic (s.c.) injections of dexamethasone (0.3 mg/kg) or a potent synthetic glucocorticoid, attenuated the memory impairment. In contrast, injections of corticosterone (0.3 mg/kg) were ineffective. Neurochemically-induced lesions of the basolateral (BLA), but not of the central (CEA) or medial (MEA) nucleus of the amygdala blocked the modulatory effects of short-term ADX and dexamethasone administration on spatial memory. These findings are consistent with previous evidence that the dexamethasone-induced enhancement of memory for inhibitory avoidance training was also selectively blocked by lesions of the BLA. ADX also impaired acquisition performance in the water maze. Lesions of the CEA, MEA, as well as lesions of the BLA, appeared to block the impairing effects of ADX on acquisition performance in the water maze. Acquisition and retention performance were not significantly affected by BLA lesions in otherwise untreated animals. In contrast, lesions of the CEA resulted in both deficient acquisition and retention performances, and lesions of the MEA resulted in an impaired retention. These experiments further provide evidence that the BLA is a critical area involved in integrating hormonal influences on learning and memory. Supported by E.W. and L. Gerard Trust Fellowship (BR) and USPHS MH12526, NIMH and NIDA (JLM).

760.13
STRIA TERMINALIS LESIONS DO NOT BLOCK BENZODIAZEPINE-INDUCED ANTEROGRADE AMNESIA. O. Carmi* and J. McGaugh.
Center for the Neurobiology of Learning and Memory, and Department of Psychology, University of California, Irvine, CA 92717-3800.
Extensive evidence suggests that benzodiazepines (BZ) induce anterograde amnesia when administered systemically or directly into the amygdala before training. Furthermore, lesions of the amygdala block the BZ-induced anterograde amnesia. The stria terminalis represents the major input/output pathway of the amygdala. ST lesions, similar to amygdala lesions, have been shown to block the memory enhancement produced by hormonal and neurotransmitters systems, including adrenocortical, cholinergic, opiate and neuropeptidic systems. Such findings imply that ST lesions should also block BZ effects on memory. The present study examined this hypothesis in rats using two tasks, inhibitory avoidance and spatial orientation water maze tasks. Bilateral ST lesions were produced by radio-frequency current in male Sprague-Dawley rats and one week later, ten minutes prior to inhibitory avoidance testing (0.05mA, 1 s), rats received injections of saline or the BZ agonist midazolam (MDL, 1 mg/kg, i.p.). On a 48-h retention-test, the retention latencies of MDL or saline injected rats were lower than those of saline-injected rats. This BZ-induced retention impairment was not blocked by ST lesions. For the water maze experiment, rats pretreated with MDL, received five training trials to find the hidden platform. The MDL impaired acquisition. Furthermore, this BZ-induced acquisition effect was not blocked by ST lesions. On a 48-h retention-test, MDL-treated rats were still impaired with respect to their control group. These data suggest that the BZ-induced memory effects are not dependent upon intact ST. These data conflict with those of many previous studies examining drug effects on learning and memory on animals with ST lesions. A possible explanation for this discrepancy is that BZ-induced amnesia is also due to an impairment in acquisition rather than modulation.
Research supported by USPHS MH51256 grants (NIMH AND NIDA) to JLM and MH14599 (NIMH and NRSA) to OC.

760.14
VA Medical Center and University of South Carolina, Columbia, SC 29208.
We reported at last year’s meeting that unilateral lesions of the central nucleus of the amygdala (ACN) and medial prefrontal cortex (mPFC) on opposite sides of the brain diminished but did not prevent acquisition of conditioned brazardiurn. These data seemed to suggest separate and parallel control of conditioned brazardiurn by the amygdala and mPFC. However, connections between mPFC and the amygdala originate not in the ACN (except for its terminals in the ventral aspects) but in the basolateral (BL) and lateral (L) nucleus. Thus, in the present experiment unilateral lesions were made on opposite sides of the brain centered on the mPFC and the Bl and L nuclei of the amygdala. One session of differential heart rate (HR) conditioning was administered, in which tones served as CSs and paraorbital shock served as USs. Although large bilateral lesions of the amygdala completely abolished conditioned brazardiurn, unilateral lesions of the mPFC and amygdala on opposite sides of the brain again diminished but did not prevent occurrence of the decelerative HR conditioned response.
Supported by VA Institutional Research Funds.

760.15
EXCITOTOCIC LESIONS IN THE BASOLATERAL AMYGDALA SELECTIVELY ABOLISH DELAYED CONDITIONED TASTE AVersions IN RATS. M. Fevetc*; S. Mannet* & D. Mitchell.
Dept. of Psychol., Loyola Marymount Univ., Los Angeles, CA 90024.
We previously demonstrated that the lateral amygdala (BLA) plays a role in mediating taste aversions. To further investigate the role of the BLA in the mediation of taste aversion, we lesioned the BLA and examined the effects of lesion on conditioned taste aversion. We found that lesions of the BLA selectively ablated conditioned taste aversions in rats. Lesions of the basolateral amygdala, but not the central or medial amygdala, selectively ablated conditioned taste aversions. These results are consistent with previous studies that have shown that the BLA is involved in the mediation of conditioned taste aversions.

760.16
INVOLVEMENT OF THE PERIAQUEDUCTAL GRAY IN FEAR-POTENTIATED STARTLE. D.L. Walker* and M. Davis.
Dept. of Psychiatry, Yale University Sch. of Med., New Haven, CT 06508.
The acoustic startle response is reliably enhanced when elicited in the presence of a cue previously paired with moderately intense footshock (i.e., fear-potentiated startle). Interestingly, startle with higher intensity footshocks produces relatively poorly potentiated startle. Because recent evidence suggests that particularly aversive stimuli may activate the periaqueductal gray (PAG) and that behaviors associated with di-PAG activation may be incompatible with startle, we hypothesized that stimuli paired with higher intensity footshocks would fail to potentiate this response and interfere with the expression of fear-potentiated startle. To test this hypothesis, two experiments were conducted.
In Experiment 1, rats were trained to respond to 30 light shock pairings using either moderate (0.6 mA) or high (1.6 mA) footshocks, followed by excitotoxic lesions of the di-PAG or sham lesions. When trained at 0.6 mA, fear-potentiated startle was comparable in both sham and lesioned animals. When trained at 1.6 mA, however, potentiated startle was reliable only in lesioned rats. Baseline startle was unaffected. In Experiment II, rats trained at 0.6 mA were tested immediately after intra-PAG infusion of either distilled water or an excitatory non-toxic dose of kainic acid. Kainic acid infusions significantly disrupted fear-potentiated startle.
Together, these results suggest that the di-PAG may be activated by stimuli associated with particularly aversive events and that this activation may interfere with the expression of fear-potentiated startle.

The effects of olfactory bulbectomy on the acoustic startle reflex, fear-potentiated startle, and shock-induced sensitization of the acoustic startle reflex were examined in a series of three experiments. In Experiment 1, bilateral olfactory bulbectomy resulted in a normal sensitization of the acoustic startle reflex relative to sham operated controls. The increase in baseline startle was persistent for up to five weeks following the lesion. Bulbectomized animals showed normal acquisition of fear-potentiated startle but enhanced context conditioning relative to sham operated controls. In Experiment 2, olfactory bulbectomy resulted in an increased sensitivity to fear-potentiated startle (0.6 mA and 1.0 mA) of the acoustic startle reflex relative to sham and unoperated controls. In Experiment 3, bulbectomized animals showed shock sensitization to a shock level (0.3 mA) which did not produce sensitization in sham and unoperated controls. Taken together, these data suggest that olfactory bulbectomy results in an increased vulnerability to stressors, perhaps because of a disinhibition of the amygdala. Thus, the olfactory bulbectomy model of depression may share some similarities with other stress-induced models of depression.

LESIONS OF THE PERIBNIAL CORTEX BLOCK CONDITIONED EXCITATION BUT NOT CONDITIONED INHIBITION OF PEAR MEASURED WITH FEAR-POTENTIATED STARTLE. W.A. Poehl, R. Bakken, S. Weaver, and M. Davis, Dept. Psychology, Northern Illinois University, Dekalb, IL 60115 and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Although much is known about the neural systems responsible for the acquisition and expression of conditioned fear, little is known about the neural systems responsible for the inhibition of fear. Following a conditioned inhibition procedure in which a light is repeatedly paired with a shock (i.e., light-shock) and a light-alone compound is presented without shock (i.e., noise-light), the noise-acquires the ability to inhibit fear-potentiated startle to the light when added to the light-inhibition summation test (Poehl & Davis, 1995). Neurosci. Lett. 192:297-301.

Perinatal cortex lesions performed after light+shock training block fear-potentiated startle. However, lesions performed in the adult do not block fear-potentiation. Because of this, it is possible to use a summation test to assess whether lesions of the perinatal cortex will disrupt previously acquired conditioned inhibition. Rats were given 15 light+shock pairings on each of 2 days followed by conditioned inhibition training consisting of 5 noise-only and 15 noise-light trials on each of 5 days. Testing occurred 1 day later. All rats showed conditioned fear to the light as defined by greater startle amplitude in the presence of the light than in its absence. All rats showed conditioned inhibition of fear, defined as a reduction in fear-potentiated startle to the light when accompanied by the noise. Next, half of the rats were given perinatal cortex lesions and half were sham operated. All rats were tested 1 week later. As expected, lesions of the perinatal cortex blocked fear-potentiated startle to the light. On each of the next 5 days, the rats were retested with light+shock trials with no further conditioned inhibition training. Testing occurred 1 day later. Perinatal cortex lesions re-acquired fear-potentiated startle to the light. Importantly, the noise-conditioned inhibitor retained its ability to inhibit fear-potentiated startle to the retrained light. Although the perinatal cortex is critical for initial performance of fear-potentiated startle, it is not critical for the expression of conditioned inhibition. We have reported that lesions of the central nucleus of the amygdala also fail to prevent the expression of conditioned inhibition to a retracted light (Poehl & Davis, Behav. Neurosci. 1993). 379-387. Hence, inhibition of fear-potentiated startle is likely to occur elsewhere in the fear-potentiated startle pathway.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XII


The tuberomamillary nuclei (TM) are located in the posterior part of the hypothalamus. The neurons of this nucleus innervate extensive parts of the brain via reticular thalamic and thalamocortical systems and represent the only source of histaminergic projections in the brain. Studies are presented dealing with lateral hypothalamic self-stimulation behavior in rats following unilateral lesions in this region in the hypothalamus, which has been described as the only ipsilateral projection area of the TM. A unilateral electrolytic lesion of the TM led to an ipsilateral increase in self-stimulation behavior beginning with the first post-lesion day. Response rates continued to rise for two weeks post-lesion. In a follow-up study the effects on self-stimulation were replicated by lesioning the TM with hexamethamine, indicating that the observed dausatory effects in both experiments are based on the destruction of intracerephysic and not on fibers passing through the lesioned area. A projection area possibly involved in the observed lateralized amplification of brain stimulation reward in the CA1 region of the hippocampus. Following a unilateral isotonic acid lesion in the TM, we have observed a decrease in the number of cells on the contralateral side of the lesion. The lesion did not result in a greater resistance to extinction, thus, it is likely that the increase in response rates can be interpreted in terms of an amplification of the rewarding value of brain stimulation and not as a form of response perseveration which has been described as a result of cortical ablation.

Our results suggest that the TM together with the hippocampus exerts an inhibitory control over lateral hypothalamic self-stimulation. These results are the first to focus on an inhibitory element in the neural substrate of reinforcement.
761.3

SELECTIVITY OF SPATIAL LEARNING IMPAIRMENT IN RATS WITH LESIONS OF THE MAMMARYLL REGION Y. Skiriak, M. Perot, and P. Jackson, McGill University, Montreal, Quebec, Canada

Rats with lesions of the mammary region (MB-R), the dorsal hippocampus (DH), and thalamic arousal (OCT) were trained on a spatial conditional associative learning task in which they had to learn to respond to one of two visual stimuli depending on their location in an open field. In a second study, rats with similar lesions were trained on a place learning task which required them to locate a hidden platform in a pool of opaque water. Rats with damage to the MB-R were able to acquire the conditional associative task at a rate comparable to that of the OC animals. The H group was significantly impaired in comparison with both the MB-R and the OC groups. By contrast, both the MB-R and the H animals were slower to acquire the location of the hidden platform in comparison with the OC group. Taken together with earlier findings from our laboratory, the present results suggest that damage to the MB-R impairs the ability to learn and remember the location of spatial cues but does not affect the capacity to discriminate between particular visual stimuli, depending on the location within which they are embedded.

761.5

AUDITORY DELAYED RESPONSE TASK DEFICITS FOLLOWING MEDIAL THALAMIC AND PREFRONTAL CORTICAL LESIONS IN THE RAT. G. R. Gallistel, Dep. of Psychology, UCLA, Los Angeles, CA 90095-1563.

The direct method for scaling subjective reward magnitude as a function of response strength uses a two-level choice paradigm with concurrent variable interval schedules of reward. The rat allocates its time between a "standard" lever delivering rewards of fixed magnitude, and an "alternate" lever delivering rewards that vary in magnitude over trials. The validity of the procedure rests on the assumption that subjective reward magnitudes and relative rates of reward combine multiplicatively to determine time allocation. If this assumption is correct, a rat will equiprobe a particular reward being delivered every ten seconds to a reward half that size being delivered every five seconds. This experiment was designed to test this assumption. The results in self-stimulating rats (male Sprague-Dawley) with electrodes in the lateral hypothalamus and VTA suggest that subjective reward magnitude and rate of reward combinations multiply to determine time allocation. The opposite assumption, that the value of either lever does not cause the rat's time allocation between levers to differ by more than approximately 61%, would have the effect of increasing the number of trials required to reach a learning criterion. This result suggests that the optimal procedure for scaling reward is to program the standard lever to deliver rewards one quarter as often as the alternate lever, and to set the reward on the standard to its maximal level. The effect of this is to prevent the net value of either lever from becoming so great that the animal's time allocation ratio differs by more than 61%.

761.6

VALIDATING THE DIRECT METHOD FOR MEASURING THE SUBJECTIVE MAGNITUDE OF BRAIN STIMULATION REWARD. M. J. L Broadie, C. I. McCallum, Dep. of Psychology, UCLA, Los Angeles, CA 90095-1563.

The direct method for scaling subjective reward magnitude as a function of response strength uses a two-level choice paradigm with concurrent variable interval schedules of reward. The rat allocates its time between a "standard" lever delivering rewards of fixed magnitude, and an "alternate" lever delivering rewards that vary in magnitude over trials. The validity of the procedure rests on the assumption that subjective reward magnitudes and relative rates of reward combine multiplicatively to determine time allocation. If this assumption is correct, a rat will equiprobe a particular reward being delivered every ten seconds to a reward half that size being delivered every five seconds. This experiment was designed to test this assumption. The results in self-stimulating rats (male Sprague-Dawley) with electrodes in the lateral hypothalamus and VTA suggest that subjective reward magnitude and rate of reward combinations multiply to determine time allocation. The opposite assumption, that the value of either lever does not cause the rat's time allocation between levers to differ by more than approximately 61%, would have the effect of increasing the number of trials required to reach a learning criterion. This result suggests that the optimal procedure for scaling reward is to program the standard lever to deliver rewards one quarter as often as the alternate lever, and to set the reward on the standard to its maximal level. The effect of this is to prevent the net value of either lever from becoming so great that the animal's time allocation ratio differs by more than 61%.

761.7


A two-trial memory task, based on place or object exploration in a Y-maze, was developed to study memory in adult and aged rats (Mayo et al., Brain Res., 588, 1992). This paradigm avoids the use of electric shocks or deprivation that may have non-specific effects, and the task does not require learning of a rule. A number of behavioral parameters in several animals could be recorded automatically. Recognition is determined by both the type of information (place vs. object) and by the inter-trial interval (recognition retention time). For place exploration, recognition was still present at the 6h inter-trial interval whereas recognition in object exploration only appeared to last for about 4h in 2-month-old rats. Memory recognition in old rats (18 months) was clearly affected by increasing the retention time. Recognition also depends on the environmental context as demonstrated by the following results: 1) place recognition remains intact in spite of an inter-trial interval (12h) at old (18 months) vs. normal (3 months) age. This significant effect was found in young and old animals. 2) object recognition is impaired when the environmental context is changed between the two trials. 3) Recognition is impaired in animals trained in a novel environment. This is of particular interest in the light of the dual mechanism suggested to be involved in recognition i.e. determination of stimulus familiarity and stimulus identification which involves retrieval of relevant contextual information processing. It is also noteworthy that contextual information processing alterations are at the basis of amnesia and memory deficits. In conclusion this memory task combines simplicity, rapidity, sensitivity and high specificity and should be useful to neuro- and psychophysicologists.

761.8

SAME-SESSION ANALYSIS OF WORKING AND REFERENCE MEMORY IN THE RAT: PROCEDURE, W. L. Wilson*, S. A. Brachmiller, M. L. Ellin, Z. Benezra, and G. Zilles, Dep. of Psychological Sciences, Indiana-Purdue University, Fort Wayne, IN 46805 USA.

Eight female Sprague-Dawley rats were trained successively in two different behavioral tasks: spatial alternation and visual discrimination in a phi-maze. In spatial alternation rats were rewarded for choosing the arm not used on the previous trial to reach a criterion of five consecutive correct responses. In visual discrimination rats were rewarded for selecting the lighted arm, the position of which varied randomly. Rats next experienced these two tasks on alternate days, each test occurring in the same session, yielding a behavioral session requiring the use of both reference (visual discrimination) and working (spatial alternation) memory. In this phase in which both tasks were employed, the rat was rewarded first for selecting the lighted arm of the maze, then for selecting the opposite arm, and so on. Thus the continuous arm effect changed following each reinforcer. Each daily session lasted until the rat received 41 reinforcers (an initial free reinforcer, and 40 earned via the selection of the appropriate arm of the maze).

Rats acquired the spatial task first, and then the visual task. When they did the visual discrimination task (means: 8.88 vs 13.00 sessions to reach the criterion of 3 successive sessions completed in fewer than 50 trials; p < 0.1), probably reflecting both the tendency to alternate spontaneously, and an initial bias to select the dark arm of the maze in the visual task. When both tasks were combined, rats performed equally well on the working and reference memory tasks in Sessions 1 - 5 (means: 23.33 [working] vs 22.63 [reference] trials to receive 20 reinforcers). Performance on the reference memory task decreased significantly below that of the working memory task (means: 25.23 trials vs 15.95 trials; p < 0.01). This procedure will serve in future studies examining the effects of amnesic or nootropic drugs. (Except for presenting author, order of authors is alphabetical.)
SEVENTY-ONE


If separable neural systems exist for the perception and discrimination of different temporal intervals, then experience with one task involving discrimination of one type of stimuli should have no effect on rate of learning of a task requiring discrimination of stimuli of similar duration, and vice versa. Rates of learning will be assessed for rats trained on temporal discrimination tasks in a standard operant chamber. In the first experiment, rats will be trained to discriminate between white noise stimuli in either a short range (SR: 200 vs. 800 msec) or long range (LR: 20 vs. 80 sec). A correct response will be defined as the number on one lever corresponding to the shorter stimulus in each task (200 msec or 20 sec) or a response on the other lever for the longer stimulus (800 msec or 80 sec). After training to asymptote, the two groups (SR and LR) will then be trained to asymptote on the opposite task (LR and SR) with lever mapping reversed for half of each group. If separable neural systems exist for the short and long stimulus tasks, then the rates of learning for all mapping groups should be the same, indicating that experience with SR timing had no effect on learning a discrimination involving LR timing, and vice versa. The second experiment will be identical to the first, except that the groups will be trained to a medium range (MR: 2 x 8 sec) discrimination task. If the SR cerebellar mediated timing system is limited to perception in the miliseconds range, as our animal data suggest, then there should be no difference in rate of learning for the two mapping groups of rats shifted from SR to MR. Facilitation would be expected in rats shifted from LR to MR with the same mapping as a LR time, responsible for learning in the second-cue domain, is involved in both tasks.

SEVENTY-TWO


Many previous studies have used the Morris water maze as a tool to investigate the cognitive functions involved in spatial learning (e.g., Morris, et al., 1982; Sutherland et al., 1987; Kelly & McVey, 1988; Whishaw, 1991). Although some of these studies have indicated that passive path integration is necessary to learn the maze, definitive data demonstrating rats' ability to rely solely on the learned relationships between various landmarks in solving the task, none of them have explicitly examined the role path integration plays in learning to locate the platform. Path integration is a process by which the animal learned the water maze.

Four groups of 6 Long-ear rats were used in training. In the 1st, a 91 m diameter water tank containing a partially submerged hidden platform. The rats were transported from their cages to the tank, either on a wooden board in a straight path (Groups 1 & 3) or in a black box in a circular path (Groups 2 & 4). Groups 1 & 2 were left on the platform for 30 min before being released onto the tank. Groups 3 & 4 were given a total of 17 min placement on the platform before the training began, and no placement on the platform before releasing them into the tank. Latency time to arrive at the platform, the most time spent and number of entries per an annulus zone surrounding the platform were recorded to assess the rats' performance. The rate at which the discrimination procedure (Groups 2 & 4) scored significantly lower on all three measures than rats not subjected to disorientation (Groups 1 & 3).

The results suggest that in the water maze, rats navigate by employing a variety of mechanisms simultaneously, including integrating their passive transportation from one location to another. It is not clear how, or how much, each mechanism contributes to successful navigation, but attention should be given to the procedures used in transporting the animal from its home cage to the task, especially when using this paradigm to test for the functional integrity of underlying spatial mechanisms.

SEVENTY-THREE


Vector voting and vector averaging are competing hypotheses about how animals use landmarks to reach a goal. A related issue is how cues from multiple reference frames interact when the landmark array moves within a larger, stable environment.

Six male gerbils were taken together and trained a task described by Collett, Cartwright, and Smith (J. Comp. Physiol., 1986). A sunflower seed was buried at the center of an equilateral triangle defined by three identical cylindrical landmarks. During training this landmark array was translated after every three trials, but not rotated. Once the animals reached criterion, no-seed probe trials were interspersed with the training trials. The probe conditions were: (a) normal array, (b) one landmark removed, (c) two landmarks removed, (d) one landmark displaced to twice the normal distance from the goal, and (e) entire array rotated by 180°.

Cumulative 2D spatial histograms of search effort closely resembled those of Collett et al. except for the (d) probe, where our animals appeared confused. Performance on the (b), (d), and (e) probes was in agreement with the hypothesis that gerbils predict goal locations by tallying votes from individual landmarks, rather than vector averaging. We developed a computer program to analyze the animals' search behavior. Histograms from multiple trials are first aligned based on the landmark array, and searching behavior is allocated to discrete bins. Differences between groups of gerbils or trials by examining the x-scapes of specific species, we can also demonstrate quantitatively the gerbils' preference for certain locations over others, allowing us to compare the predictions of competing hypotheses.

Probes (b), (d), and (e) are evidence for the (a) case, indicating the gerbils were aware of perturbations to the landmark array. The experiments also revealed that most probe results reported by Collett et al. can be obtained without any effort to eliminate stable room cues.
761.15
SPATIAL ALTERNATION IS NOT FACILITATED BY TRANSECTION OF THE CORPUS CALLOSUM. D.P. Crowne and S.P.C. Gray, Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada. N2L 3G1.

In The Psychology of Left and Right, Corballis and Boase proposed that mutual sharing of mirror-image information by the cerebral hemispheres confounds the discrimination of left and right stimuli, so that an anterior and interacting hemispheres will learn mirror-image tasks more slowly than one with a divided brain. Discriminating mirrored left and right responses should be similarly affected. Noonan and Axelrod examined the latter, comparing splintmam and intact rats on a conditional spatial discrimination. Their splintmam animals were markedly better. Here, we studied another, more basic spatial problem that requires mirrored left and right responses: delayed spatial alternation. Splinterm (n=11) and control (n=15) rats were trained on spatial alternation in a water T-maze similar to Noonan and Axelrod's and tested for 800 trials. The mean trials to criterion of controls was 449.05, of splintmam 692.73, (F(1, 18)=9.74, p<.01). All controls learned; 7 splintmam failed to learn. It appears that only some mirror-image spatial tasks are better performed by splintmam animals, perhaps those involving a conditional cue. The more general effect of callosum section on spatial learning is a severe deficit, earlier shown on allocentric tasks and now on an egocentric one.

761.16
Thiamine Deficiency in 16 month old rats versus 3 month old rats: Behavioral and pathological differences. L.M. Stycus**, R. Castillo, & P.J. Langlais, Department of Psychology, SUNY-Binghamton, San Diego State University, and VA Medical Center San Diego. 92161.

To examine age-dependent differences in the neuropathology and behavioral consequences of Pyridoxine-Induced Thiamine Deficiency (PTD) a group of older rats (16 months) than those used in previous studies (3 months) were placed in and the older than the younger water maze and tested to either a correct or incorrect criterion, re-trained on the water maze, and then tested on an acoustic startle task. A standard bout of PTD produced a higher mortality rate (69%) in these older rats than previously observed in young rats (20%). Furthermore, after the reversal of PTD treatment, the older rats also took twice as long (4 vs 2 weeks) to regain weight lost during treatment, relative to young PTD treated rats. When re-tested on the Morris water maze, the older PTD rats had longer latencies to reach the platform compared to their last pre-training session or relative to the older control rats on re-acquisition. This "regrade loss" was not observed in a previous study using 3 month old PTD treated rats. There were no groups difference observed on the acoustic startle task. The neuropathology produced by PTD treatment in the older rats is different from what has been seen in young PTD-treated rats. Thus, the age at which a rat is exposed to PTD treatment appears to influence both the behavioral and pathological consequences.

This research was supported by a VA Merit Award to PIL.

761.17
EFFECTS OF NEONATAL OXYGEN DEPRIVATION ON DEVELOPMENT OF SPATIAL LEARNING IN RATS. R.L. Harman* and C.R. Armit, Developmental Neuropsychology Lab, Dept. Psych., Washington University, St. Louis, MO 63108.

Human neonatal oxygen deprivation has been related to a wide range of developmental disabilities, including cerebral palsy, mental retardation and behavioral disorders. The present study determines the effects of neonatal oxygen deprivation on the development of spatial learning and memory in the albino rat. At postnatal ages (days) PN 0 to PN 7, pups were subjected to 3 consecutive periods of oxygen deprivation (gassing criteria) separated by 30 minutes designed to re-oxygenate.

Beginning on PN 18, control and oxygen-deprived (OD) rats were tested in a Morris water maze for 10 consecutive days. Three conditions were tested: distal/spatial (invisible escape platform in a fixed location), proximal/visual (visible platform in a fixed location), and random (invisible platform placed in random locations). Each of the 10 testing days consisted of 12, 60-second acquisition trials (the maze contained an escape platform), and a 60-second retention trial (the maze did not contain an escape platform). For acquisition trials, latency to enter the maze quadrant containing the platform, and latency to find the platform, were recorded (maximum score = 60 sec). For the retention trials, latency to enter the quadrant that previously contained the platform, and total amount of time spent in that quadrant, were recorded (maximum score = 60 sec).

During acquisition faster than control rats under the distal/spatial condition. During retention trials, however, control rats in the distal/spatial condition spent more time in the quadrant that previously contained the platform than did OD rats. It appears that neonatal oxygen deprivation may differentially affect acquisition and retention of spatial abilities. Acquisition escape latency may have been faster for OD rats via hyperactivity, whereas retention for the platform's location was adversely affected. These results may be related to the development of glial/aminergic NMDA receptor excitotoxicity.

761.18

Despite the increasing evidence that low-level lead (Pb) exposure lowers IQ, relatively little is known about the specificity of the impairment. This presentation will focus on 3 tasks included in an investigation designed to probe specificity of this issue. Pb-exposed S's displayed impaired inhibition of prepotent responses in a vigilance task, consistent with prefrontal cortex (PFC) dysfunction. In a win-shift radial maze task, both Pb-exposed groups were significantly impaired relative to controls when tested after an 8-hr retention interval; at shorter delays, they were either unimpaired or superior to controls. This pattern demonstrates a specific impairment in declarative memory, consistent with the evidence that Pb exposure alters hippocampal LTP and NMDA receptor function. This latter task and a matching-to-position task provided evidence that developmental Pb exposure produces a lasting increase in the responding value of reinforcers. The increased incentive and impaired response inhibition may both be related to altered dopamine (DA) activity, in nucleus accumbens and in PFC respectively. The evidence that DA activity in PFC inhibits accumbens DA activity suggests that the observed alternations in executive function and incentive motivation may be linked. It is notable that changes in these two functional domains are also seen following prenatal alcohol exposure and in ADHD.

Supported by grants from NIEHS (ES-05959, ES-07457) and the March of Dimes Birth Defects Foundation (12-0730).

761.19
PLACE RETENTION, EXPLORATION, HABITUATION, AND SEROTONIN IN THE RAT. G. Wirstein, P. Pliangla, E. Melinou, and J. Mogensen, Lab. of Neuropharmac. Dept. of Pharmacology, University of Copenhagen and Rigshospitalet-S102, The University Hospital, Copenhagen, Denmark.

A selective lesion of the serotonergic system was made by intracerebroventricular injections of 0.1mg 5,7-dihydroxytryptamine in 1Iq saline 0.1% acetic acid per side. Selectivity was assured by pretreatment with norfinnmine and desmipramine. Postoperatively, habituation of locomotion in an activity cage, habituation of exploration in a vertical holeboard apparatus and retention of a place-learning task in a water-maze were tested.

Quantitative analysis of the serotonin reuptake test confirmed the lesion by showing a Bmax reduction of more than 90%. While habituation of locomotion was not affected by this almost complete elimination of the serotonergic system, habituation of exploration was impaired. Furthermore we found place recall to be significantly impaired. Finally, a pharmacological challenge by scopolamine (0.5mg/kg) demonstrated that the place recall of the lesioned animals was hypersensitive to muscarinergic receptor blockade.

These results emphasize the importance of serotonergic and cholinergic interactions for spatial memory - an interaction which may be of importance in Alzheimer's disease.

761.20
EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON PASSIVE AVOIDANCE BEHAVIOR IN RATS. L.C. Devos**, R. Lopez and A. Frasier, Dept. Pharmacology, Univ. Texas Health Science Center at San Antonio, TX 78284-7764. Performance in a passive avoidance (PA) task can be altered by drugs that block 5-ht-agonistic (BAR) and muscarinic cholinergic receptor systems. The amnestic effect of the muscarinic antagonist, scopolamine, in a PA task can be potentiated by blockade of BARs; blockade of amydaloid BARs may itself cause an amnestic effect. Chronic antidepressant (AD) treatment of rats results in down-regulation of BARs and this effect occurs robustly in the amygdaloid nuclei, a brain region strongly linked to the regulation of emotionality, particularly fear. Consequently, we determined whether AD treatment in combination with an acute injection of the muscarinic receptor antagonist, scopolamine, would disrupt retention on a PA task. Initial experiments conducted earlier reports in that: (1) propranolol (80 & 68 minutes), injected directly into the amygdala complex of rats trained on a PA task, produced dose dependent decreases in retention of the task, and (2) blocking BARs, by systemic administration of propranolol (10 mg/kg, i.p.), potentiated the amnestic effect of scopolamine in a PA task.

Rats were treated either phenelzine (5 mg/kg, i.p.) daily for 21 days, or desipramine (10 mg/kg, i.p., bid) for 8 days. Control animals received vehicle at corresponding times. Twenty minutes prior to acquisition training (electric footshock, 0.5 ma for 2 secs) (amphetamine, 10 mg/kg, i.p., for 4 days) each of the three treatment groups were randomly divided and given an acute injection of either scopolamine (0.15 or 0.3 mg/kg, i.p.) or saline. Retention (10 min. test) was recorded 24 hours later. All groups showed maximal or near maximal retention on the test day. There was a tendency for rats receiving chronic AD treatment prior to scopolamine injection to exhibit reduced retentions, but these differences were not significant. Chronic treatment of rats with ADs does not alter performance or potentiate the effect of scopolamine on a PA task. Only blockade amydaloid BARs with propranolol, AD-induced down-regulation of such receptors does not produce an amnestic effect in a PA task. (Supported in part by Research Funds from the VA and USPHS Grant MH 25904.)
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762.1 CHALLENGING IBOTENIC ACID LESIONING: A NEUROANATOMICAL TOOL TO ASSESS THE INTEGRITY OF FIBERS OF PASSAGE IN BEHAVIORAL-LESION STUDIES. S. Feng, R. Morris, and M. Petrides, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

In behavioral-lesion studies involving animals, ibotenic acid (IBO) is widely used as a means of disrupting brain structures. IBO is considered to be the lesioning method of choice since it destroys cell bodies but leaves fibers of passage intact. If IBO, however, is not used properly, i.e., if the concentration or the volume are too high when injected in a given area, both cell bodies and fibers of passage will be destroyed. The present project evaluates, first, whether ibotenic acid (IBO) is a reliable means of destroying a specific neural structure, and, second, whether it really leaves fibers of passage intact through the lesioned area. In 20 rats, the central nucleus of the amygdala was destroyed with IBO and the fibers that pass through this brain region were evaluated by means of an anatomical tract-tracing technique, namely HRP-WGA histochromy.

The results of this study have shown that IBO could be an ideal means of destroying cell bodies in a given brain area while at the same time sparing fibers running through that area. However, anatomical tract-tracing methods should be considered to assess the success of this technique. In light of the present results, behavioral-lesion studies would benefit from applying this reliable yet simple technique.


Seven day old rat pups received bilateral intraventricular injections of the cholinergic immunotoxin 192-192 saporin (200 ng/1.5 nl/ventricle). Four times later the immunotoxin-injected rats showed profound (88%) reduction of cholinoceptor transduction activity in the hippocampus with reductions in cortex (51%) and caudate (30%). Forebrain monoamines were unaffected except for increased NE and DA in cortex and NE, but not DA, in hippocampus. Immunohistochemical labelling of the p75 NGF receptor a marker for basal forebrain cholinergic neurons were reduced innervation of the dentate gyrus and to a lesser extent in CA2 and CA3. Despite profound reduction and abnormality of hippocampal cholinergic innervation, the treated rats showed neither a spatial memory impairment on the Morris water task, or a short term memory impairment on a spatial alternation task. (Supported by NSERC).

762.3 VISUO-SPATIAL LEARNING IN MICE EXPOSED TO PRENATAL GAMMA RADIATION. R.W.F. Vitali* (1), C. Moreira (2), M. Dunlop (3), D. L. Linden (2), and S. T. Schmidt (1). (1) Departamento de Ciências Fisiológicas, Universidade do Estado do Rio de Janeiro, R. 20550-510, Rio, Brazil (2) Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Brazil (3) Department of Psychology, University of Alberta, Edmonton, Canada.

Exposure to gamma radiation at 16 days of gestation (E16) produces in the progeny extensive shrinkage of the occipital cortex and disruption of the laminar pattern of the hippocampus. This investigation was designed to study visuo-spatial learning in irradiated animals. Pregnant females were exposed to a gamma source at E16 with a total dose of 35Gy. Spatio-temporal task was a box (n = 28 irradiated and n = 30 non-irradiated) was assessed in the Morris water maze task. This behavioral testing was conducted on two consecutive days, with each mouse receiving 5 trials per day. After 1 week, controls (n=10 irradiated, n=10 non-irradiated) were also tested in the Lashley III Maze. In this test each animal received 10 trials, one trial per day. Analysis of the Morris water maze showed that there was not a significant difference between irradiated and non-irradiated animals on the first trial of the first day. On the subsequent trials, the time to reach the hidden platform was consistently greater in the irradiated animals as compared to the normal mice. Similarly, analysis of the Lashley III Maze showed a significant difference between the two groups after the fourth trial. After this trial, normal mice learned the task whereas the irradiated animals never reached the learning criterion. These data show a deficit in the visuo-spatial learning of the irradiated animals and may be a consequence of the early lesion in the hippocampus.

762.4 NEONATAL VENTRAL HIPPOCAMPAL LesIONS IMPAIR SPATIAL LEARNING AND MEMORY IN THE RADIAL-ARM MAZE AND ATTENUATE RESPONSE TO THE NITRINIC ANTAGONIST MECAMYLAMINE. R.A. Chambers, J.P. McEvoy and E.D. Levin*. Neurobehavioral Research Laboratory, Psychiatry Department, Duke University Medical Ctr., Durham, NC 27710 USA.

In a rat model of schizophrenia developed by Lipska et al. (Neuropsychopharmacology 9:67-75, 1993), ibotenic acid lesions are made in the ventral hippocampal formation on Day 7 after birth which results in locomotor hyperactivity with an onset during adolescence. We studied the cognitive effects of this lesion by examining spatial learning and working memory in the radial-arm maze. Testing began on Day 40 with 6 lesioned and 14 sham operated male and female Sprague-Dawley rats. The lesioned rats had significantly lower scores (p<0.025) lower accuracy and latency compared to controls. Testing continued to Day 200, with nicotine (0.1-0.4 mg/kg), mecamylamine (2.5-10 mg/kg) and scopolamine (0.4-1.6 mg/kg) were administered. Lesioned rats showed a significantly attenuated response to the nicotinic antagonist mecamylamine (p<0.025), suggesting that this system was less critical in lesioned rats than controls for performance of the memory task. These results demonstrate that in addition to hyperactivity, ventral hippocampal lesions on Day 7 cause long-term deficits in spatial learning and working memory function. This may provide a model for the cognitive deficits seen in schizophrenia. (This research was supported by the National Alliance for Research on Schizophrenia and Depression.)

762.5 ACUTE INJECTIONS OF QUINOLINIC ACID CAUSE DOSE-DEPENDENT SPATIAL MEMORY DEFICITS IN RATS. G.L. Dunbar*, M.C. North, K.L. Hall, and J. Dong. Brain Research Laboratory, Department of Psychology, Central Michigan University, Mt. Pleasant MI 48859

Previous work has indicated that acute bilateral injections of quinolinic acid (QA) can mimic some of the neurochemical conditions observed in Huntington's disease and can produce profound learning deficits in a Morris water maze task. It was not previously known whether acute bilateral injections of QA could cause memory deficits in a well-learned spatial memory task. The present study investigated this possibility. Six groups of rats were given acute bilateral intrastriatal injections of QA at 0, 100, 200, 375, and 500 nmol following acquisition of a radial-arm water maze spatial learning task. Although rats with the higher doses of QA took significantly longer to reacquire the task, all rats eventually were able to relearn the task. These results indicate that while acute bilateral intrastriatal injections of QA can disrupt spatial memory in rats, the memory deficits produced are not permanent and are not as profound as those observed in acquisition tasks.

762.6 THE HIPPOCAMPUS CONTRIBUTES TO SPATIAL LEARNING AND MEMORY IN RATS WITH Fimbria/Forexh lesionS. D. Haneman* and R. W. Shimp. Dept. of Psychol. Univ. of Victoria, B.C., Canada, V8W 3P5

Fimbria/ fornix (FF) lesions consistently produce impairments in a variety of spatial paradigms but do not prevent spatial learning entirely since FF-lesioned animals can utilize spatial solutions in such tasks when given extensive training. Though the anatomical substrates of this preserved capacity are unknown, a plausible hypothesis is that the hippocampus continues to subserve some of its normal function since both its internal circuitry and connections remain largely intact after these lesions. The present study investigated this hypothesis by examining the role of the hippocampus major cortical input/output pathway, the perforant pathway (PP), in spatial learning and memory after FF lesions. In Experiment 1, rats (females) were subjected to FF lesions, combined FF and PP lesions, or sham operations (SH) were tested for acquisition of a constant platform location in the Morris water maze. In agreement with previous findings, PP lesioned rats were impaired in the rate of acquisition of the platform's location but did not prevent it. Concurrent PP lesions aggravated this impairment but still permitted some spatial learning. In Experiment 2, these same rats received a PP lesion or a combination of QA lesions and PP lesions (QA/PP) and were then tested for memory function. QA lesions produced memory impairment in a PP-lesioned rats and produced a more modest deficit in sham-operated rats. Further, both groups' performance improved to the levels of their respective controls (FF/SH, SH/SH) with further training. No lesion effect was observed as both PP/FF and FF/FS/SH performed at comparable levels. Our results suggest that the QA lesions contribute to residual spatial learning and memory abilities after FF lesions. However, since ventral portions of the FF were spared by our lesion procedure, it cannot be determined whether specific hippocampal structures were responsible for residual function following combined FF-PP lesions.

The volume and location of hippocampal (HPC) tissue required for normal spatial learning in rats was investigated. Iontogenic acid was used to make bilateral, symmetrical lesions of 20-100% of HPC volume (Fig. B-D), with the spared tissue as 100% at both the dorsal and ventral pole of HPC. Spatial watermaze learning was more efficient when the residual tissue was in the dorsal as than in the ventral HPC. Even small transverse slabs of HPC (ca 28% of total) disrupted spatial learning provided they were in the dorsal pole, whereas 50-60% had to be spared to achieve normal spatial learning with blocks starting from the ventral pole (1°). Acetylcholinesterase staining, population spikes and synaptic plasticity in the remaining dorsal and ventral slabs were inside normal ranges. Thus, HPC-dependent spatial learning only requires the integrity of only a minislab of HPC tissue.


Prior studies of postmortem tissue from the hippocampal formation (HF) found lesions in the dentate-hilar area to be particularly correlated with Alzheimer’s dementia, and prior animal work has suggested that lesions to this area might be particularly disruptive of learning and memory in the rat. As a test of this hypothesis, 30 male rats 50 days of age were trained to press a lever to obtain sucrose pellets on an FR3 schedule only when a stimulus light was off. At 80-82 days of age 10 animals received bilateral injections of 0.2 ml isotonic acid (200 mM) in the anterior and posterior dentate gyrus/hippocampal (DG/H) region, 10 animals injected nothing. Surprisingly, accuracy for the task was not altered for the lesioned group compared to the control group (p<0.03), which suggests that the DG/H is not involved in the learning and memory of this task.


Lesions of hippocampal connections such as the fimbria-fornix, impair performance on tasks that require the formation of a spatial-cognitive map, but spare the ability to solve non-spatial tasks. A number of studies on the radial-arm maze have found that rats with fimbria-fornix lesions actually perform better than intact rats on various non-spatial tasks. However such has never been reported in the Morris water maze, perhaps because the non-spatial tasks previously used have been too easy.

The current study compares performance of intact and fimbria-fornix lesioned rats on a spatial task and a relatively difficult non-spatial trajectory task in the Morris water maze. Subjects were started from four different locations on each of seven days of training. In the spatial task, the platform location remained fixed with respect to the training room, regardless of the start location. In the trajectory task, the platform location remained fixed with respect to the start location (i.e. to the left of the start position), regardless of the position in the room. The results show that lesioned animals were impaired on the spatial task (p<0.05) but performed significantly better than controls on the trajectory task (p<0.05).

The results suggest that the formation of a spatial cognitive map by the intact animals placed them at a disadvantage on the non-spatial trajectory task. The fimbria-formin lesioned rats could not form such a map, and therefore did not suffer the same disadvantage on the trajectory task.


The ventral hippocampus (VH) is a part of a neural circuit which includes the prelimbic region (PL) of the medial prefrontal cortex and the nucleus accumbens (N.Acc.). The purpose of this investigation was to determine the unique contribution of the VH in spatially-mediated behaviors. Well trained rats with lidocaine-induced lesions of the VH made more revisit errors (relative to control rats of the same group), whereas rats with lesions in the PL made fewer. The DA antagonist SCH-23390 (0.05, 0.5, 0.5, 0.5) increased the number of revisit errors, whereas SCH-52022 (0.05, 0.5, 0.5, 0.5) decreased the number of revisit errors, but these treatments did not affect performance in spatial memory tasks. These results suggest that the VH is involved in the initiation of spatial information and may be responsible for guiding behavior on the maze.

A delayed-match-to-sample for spatial location task on the dryland version of the Morris water maze was utilized to assess spatial interference in rats. During the task’s study phase an object which covered a baited food well was randomly positioned in one of six possible spatial locations. Rats of 2.5-3.5 g were placed from the front wall or white light presented from the ceiling. Choice stimuli included red, blue, green and yellow solid colors presented on two side keys. Correct choices were followed by 2-s access to reward, and incorrect choices were followed by a 15-s ITI. Interference was studied using a duration matching-to-sample (MTS) procedure. Pigeons were initially trained on two duration MTS problems in acquisition. Samples consisted of 7 and 15-s durations of red, green and blue lights. Interference could be produced by 2-s access to reward, and incorrect choices were followed by a 15-s ITI. Before and after receiving bilateral aspiration lesions of the hippocampus, pigeons were tested with normal (nonprobe) and probe trials in both the subjective timing and short-term memory tasks. No probe trials for both test sessions consisted of red and white samples immediately followed by appropriate choice stimuli. Test sessions of subjective timing included a random presentation of 40 nonprobe and 8 probe trials. These probe trials consisted of red or white samples presented for 3-, 5-, 7- or 9-s. Compared to pre lesion performance, pigeons were more likely to respond 'long' following hippocampus lesions, but this effect differed as a function of sample color. Test sessions of short-term memory included a random presentation of 24 nonprobe and 24 probe trials. These probe trials also involved 2- or 10-s presentations of red or white light; but, 0-, 1-, 5-, 6-, or 12-s delays were imposed between signal offset and choice stimuli onset. Pre- and post-lesion accuracy was disrupted on long samples following the retention interval but this effect was not found using short samples. Results will be discussed in terms of changes in temporal processing following a disruption of normal hippocampus functioning.

762.15 THE EFFECTS OF HIPPOCAMPAL LESIONS IN PIGEONS: VISUAL DMS WITH INTERFERENCE AND SPATIAL MEMORY. M. Columbus* and S. Caskey, Department of Psychology, University of New Orleans, New Orleans, LA.

Previously (SN Abstracts, 20(102)) we reported that hippocampus and area parahippocampalis (Hp-APH) lesions in pigeons had no effect on the postoperative retention or acquisition of a visual DMS task, or the postoperative acquisition of a concurrent discrimination task. Given that Hp-APH lesions cause deficits in homing behavior, these results suggested that the hippocampus in pigeons functions mainly to support spatial information, rather than visual information. A number of primate studies have reported that the effects of hippocampal lesions are exacerbated under conditions of interference. In this current study we explored whether visual DMS behavior in pigeons was impaired by testing with two forms of interference. The same ten subjects that served in the previous report served in the current study. Five had received bilateral Hp-APH lesions and five served as unoperated controls. In Experiment 1, we examined the effects of proactive interference on visual DMS behavior by testing the pigeons with either a 15 sec or 1 sec interval (ITI). Confirming previous studies, reducing the ITI led to an impairment of performance. However, both control and Hp-APH pigeons were equally affected. In Experiment 2, we examined the effects of retroactive interference by interjecting visual interference during the delay period. Visual interference reliably impaired performance in both control and Hp-APH pigeons. Again, however, both control and Hp-APH pigeons were equally affected. In Experiment 3, we examined the effects of Hp-APH lesions on the acquisition of a open-field radial maze task. Of the five birds tested to date, three Hp-APH pigeons required 38, 41, and 47 days to learn the task, whereas two control pigeons learned the task in 6 and 14 days. These results provide further evidence that the pigeon hippocampus is important for the processing and retention of spatial rather than visual information.


The role of the avian hippocampus in subjective timing and short-term memory of temporal events was studied using a duration matching-to-sample (MTS) procedure. Pigeons were initially trained on two duration MTS problems in acquisition. Samples consisted of 2 and 10-s durations of red, green and blue lights presented from the front wall or white light presented from the ceiling. Choice stimuli consisted of red, blue, green and yellow solid colors presented on two side keys. Correct choices were followed by 2-s access to reward, and incorrect choices were followed by a 15-s ITI. Before and after receiving bilateral aspiration lesions of the hippocampus, pigeons were tested with normal (nonprobe) and probe trials in both the subjective timing and short-term memory tasks. No probe trials for both test sessions consisted of red and white samples immediately followed by appropriate choice stimuli. Test sessions of subjective timing included a random presentation of 40 nonprobe and 8 probe trials. These probe trials consisted of red or white samples presented for 3-, 5-, 7- or 9-s. Compared to pre lesion performance, pigeons were more likely to respond 'long' following hippocampus lesions, but this effect differed as a function of sample color. Test sessions of short-term memory included a random presentation of 24 nonprobe and 24 probe trials. These probe trials also involved 2- or 10-s presentations of red or white light; but, 0-, 1-, 5-, 6-, or 12-s delays were imposed between signal offset and choice stimuli onset. Pre- and post-lesion accuracy was disrupted on long samples following the retention interval but this effect was not found using short samples. Results will be discussed in terms of changes in temporal processing following a disruption of normal hippocampus functioning.

762.17 PLACE LEARNING ON THE MORRIS WATER TASK DOES NOT REFLECT GA SYNAPTIC DENSITY CHANGES ACROSS THE ESTROUS CYCLE. S.G. Warren* and J.M. Juraska, Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Synaptic density and long term potentiation (LTP) hippocampal GABA stratum radiatum vary significantly across the rat estrous cycle. The current study examined the behavioral implications of these rapid neural changes across the estrous cycle. While previous studies have suggested that spatial learning varies between the behavioral estrous and diestrus phases of the cycle no attempts were made to minimize the stressful aspects of the task, even though the stress response varied significantly across the estrous cycle. In the current study females in each phase of the estrous cycle and males were trained on a place version of the Morris water task, with each female's training repeated over one phase of the cycle. To reduce the stress involved with performance on this task all animals received extensive handling and pre-training (in a different room) in a small well-lit room and appropriate platform results indicated that performance varied across the estrous cycle. On several measures females in estrus were similar to males, while rats in diestrus were similar to males, with females in proestrus, with females in proestrus, performing more poorly than males and females in estrus. The anatomical and LTP changes across the cycle do not correlate with the behavior. Supported by NSF#89139045 to JM and HD07333 to SGW.


The University of Michigan, Ann Arbor, MI 48109-0402.

Damage to the hippocampus (HPC) produces greater place navigation deficits in females than males. We also find that female animals with HPC damage are more resistant to therapy and more disruptive than males. This study examined the influence of the estrus cycle on spatial learning in the presence and absence of HPC damage. We hypothesized that the stage of estrus in the test day significantly affects spatial learning on subsequent days as prior work has shown that the majority of learning occurs between the first and second day of behavioral testing. Adult female (N = 12) rats received bilateral HPC lesions or sham surgery. Upon recovery, all animals were given 4 days of training on the Morris water task. Daily vaginal smears determined the stage of estrus cycle. As expected, behavioral deficits were revealed that animals with bilateral damage were markedly impaired when compared to controls (p<.05). Stage of the estrus cycle did not significantly affect performance in controls. In contrast, lesioned animals in proestrus (proestrus and early estrus), high estrus (day 1) showed no effect. Low estrus (day 3) or mid estrus (proestrus and mid estrus) were most severe, as evidenced by shorter swim times on the first 2 days (day 1: X = 71.06 ± 13.98 vs X = 114.09 ± 19.30, p = .10 and day 2: X = 39.25 ± 14.91 vs X = 91.03 ± 14.61, p<.05). The groups continued to differ across all 4 test days. The extent of HPC damage did not differ between the two lesioned groups.

Qualitative analysis of swim paths revealed that lesioned animals in proestrus displayed more purposeful swim behavior strategies compared to the perseverative patterns of females in diestrus. These results show that animals with HPC damage quickly adapt more efficient learning strategies than those of hippocampally intact rats.
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763.1 INTERACTIONS BETWEEN BENZODIAZEPINE AND DOPAMINE RECEPTORS IN THE MODULATION OF CORTICAL ACETYLCOLINE RELEASE

Benzodiazepine receptor (BZD-R) inverse agonists have been shown to disabit cortical acetylcholine (ACh) efflux via their ability to negatively modulate GABA-mediated chloride flux in the basal forebrain. In the present experiment, the BZD-R inverse partial agonist, FG 7142, was used to test the hypothesis that the (DA) receptor in the nucleus accumbens (NA) interact with GABA/BZD-R in the regulation of cortical ACh release. Systemic administration of FG 7142 was preceded by systemic or intra-accumbal administration of selective D1 (SCH 23390) and D2 (chloropropion) antagonists, and the neurolepatic haloperidol. ACh efflux in the frontal cortex was measured in awake animals using microdialysis. FG 7142 (1.0 mg/kg) increased cortical ACh efflux. This increase was dose-dependently attenuated by systemic administration of the D1 antagonist (0.1, 0.3 mg/kg) or haloperidol (0.15, 0.9 mg/kg). The D2 antagonist, chlorpropiol (10 mg/kg), however, had no effect on stimulated ACh efflux. Haloperidol administered into the NA (10, 100 μg/kg) appeared to block FG 7142-stimulated cortical ACh efflux, whereas equilimus doses of SCH 23390 into the NA did not. Neither drug had an effect intracortically, via the microdialysis probe, blocked FG 7142-increased ACh efflux. These results suggest that the well-known ability of FG 7142 to stimulate DA release may contribute to the induction of cortical ACh release. This DA-mediated effect does not occur locally within the frontal cortex and the site of action of D1 antagonists (i.e., non-accumbal) and neuroleptics (i.e., accumbal) can be dissociated.

763.2 BiDIRECtIONAL TRANS-SYNAPTIC MODULATION OF CORTICAL ACETYLCOLINE IN INTACT AND 192 IgG-SAPORIN-LESIONED RATS.
L. J. Pelletier, R. M. Hori, and J. F. Pelletier.

Loss of cortically-projecting basal forebrain cholinergic neurons correlates with the cognitive decline associated with Alzheimer’s disease. Trans-synaptic modulation of the remaining cholinergic neurons may prove therapeutic for augmenting cortical acetylcholine (ACh) release in close temporal contiguity with the activating demands of cognitive processing. Liganids that modulate the GABA/Benzodiazepine receptors and/or the (D2) complex represent a potential site for this type of modulation. The present study utilized in vivo microdialysis in awake rats to test the effects of positive and negative modulators of GABA-mediated chloride flux on cortical ACh efflux in vivo. Drugs that augmented or reduced the basal forebrain dischareflessen with cortical microinjections of the amnestic 192 IgG-saporin. Lesioned animals exhibited a 50-60% reduction in basal cortical ACh efflux but responded to the GABA positive modulator, BZD-R partial inverse agonist FG 7142 (8.0 mg/kg) with a stimulation of ACh efflux similar in magnitude to that seen in intact rats (100-200% above baseline). These findings were confirmed with another negative modulator of the NA, the BZD-R inverse agonist ZK 93 426. Acute systemic administration of the positive GABA modulator ethanol (1.0 g/kg, ip) produced an initial increase, followed by a decrease in cortical ACh efflux in intact animals. The effects of localized perfusion of ethanol and its effect on cortically dischareflessed rats will also be discussed.


The different cognitive functions of the major cortical afferent systems have remained untested. An open procedure that required the animals to detect visual signals (presented for 25 - 500 msec) and to discriminate positive and non-signals was used to assess the role of forebrain noradrenergic afferents in behavioral vigilance. Measures of performance include the relative number of hits (correct and incorrect responses to signals), and correct rejections and false alarms (correct and incorrect responses to non-signals). Performance was characterized by a signal length-dependent to detect signals, and by performance decrements over time (vigilance decrement). Following the pretreatment with Pargyline (50 mg/kg), 6-hydroxydopamine was injected into the dorsal noradrenaline bundle (2 μg/μl/hemisphere). The lesion reduced the concentrations of noradrenaline in the cortex by > 90 % and in the hippocampus > 80 %. Post-surgery performance of the lesioned animals was characterized by relatively small and rapidly recovering decreases in both the relative number of hits and correct rejections. This finding contrasts with the persistent, potent, and selective decrease in the relative number of hits following 192 IgG-saporin-induced basal forebrain lesions (McCaughy et al. 1995). Thus, behavioral vigilance is likely to depend on the integrity of forebrain cholinergic, but not noradrenergic inputs.

763.4 FUNCTIONS OF CHOLINERGIC INPUTS TO VISUAL CORtICAL AREAS: EFFECTS OF VISUAL CORTICAL CHOLINERGIC DEAFFERENTATION ON VISUAL ATTENTION IN RATS.

The functions of the cholinergic inputs to visual cortical areas remain largely unknown. Likewise, the extent to which loss of cholinergic inputs to the visual cortex contribute to the impairments in visual and cognitive functions in senile dementia is not clear. Our previous research on the effects of infusions of 192 IgG-saporin into the visual cortex revealed an impairment in the acquisition, but not performance, of complex visual discriminations of variable difficulty. In the present experiment, 192 IgG-saporin (0.008 μg/μl) was infused into the primary and secondary visual cortex (two infusion sites per hemisphere) of rats trained in a visual sustained attention task (McCaughy & Sarter, Psychopharmacol. 117:340-357). This task requires the animals to detect visual signals presented for 25, 50 or 500 msec and to discriminate these signals from non-signals. Present data suggest that the partial loss of cholinergic input to visual cortical areas impairs performance, primarily by decreasing the animals' ability to detect longest signals and to correctly reject non-signal trials. These findings contrast with the selective and signal length-dependent decrease in the detection of signals in animals with 192 IgG-saporin lesions of the basal forebrain and suggest a discrete function of visual cortical ACs in visual attention.

Previous work demonstrated that infusions of the benzodiazepine receptor (BZR) agonist chloridiazoxide (CDP) or the BZR inverse agonist β-carboline β-CCM into the substantia innominata of the basal forebrain produced dissociable effects on the performance of animals tested in a behavioral vigilance task. Specifically, CDP selectively decreased the animals' ability to detect signals while their ability to correctly reject non-signal trials remained unaffected. In contrast, infusions of β-CCM selectively increased the relative number of false alarms (i.e., claims for hits in response to non-signal trials; Holley et al., Psychopharmacol., in press). Because these findings were related to the contrasting effects of BZR agonists and inverse agonists on cortical acetylcholine (ACh) efflux (Moore, Sarter, & Bruno, Neurosci. Lett., in press), and because of the transsynaptic regulation of cortical ACh efflux by acetylcholine via its effects on the acetylcholine-gABAergic projection to the basal forebrain neurons, this experiment tested the hypothesis that systemic co-administration of haloperidol (0.001 - 0.01 mg/kg) augments the attentional effects of intrabasal infusions of the BZR agonist (40 μg/hemisphere) and attenuates the attentional effects of intrabasal infusions of the BZR inverse agonist (5 μg/hemisphere).


Previous work demonstrated that systemic administration of the benzodiazepine receptor (BZR) partial inverse agonist β-carboline FG 7142 (FG) augments the cardiovascular response to nonsignal stimuli, and mimics the cardiovascular effects of a conditioned stimulus for an aversive event. Analyses of the parasympathetic and sympathetic contributions to the effects of FG prompted the hypothesis that increases in central cholinergic activity mediate the potentiation of the cardioacceleratory response to FG. Consistent with this hypothesis, the present experiments demonstrate: a) ICV infusion of the muscarinic agonist carbachol mimics the response-potentiation effects of FG; b) this effect of carbachol was blocked by ICV co-administration of the muscarinic antagonist atropine; c) ICV infusions of atropine blocked the potentialization of the cardioacceleratory response seen after ICV administration of FG, but did not alter the basal response to the stimulus; and d) 192 IgG-saporin-induced lesions of basal forebrain cholinergic neurons prevented the FG-induced potentiation of the cardioacceleratory response, without altering the basal cardiac response. These data strongly support the hypothesis that the effects of FG on cardiac reactivity are mediated via an activation of central muscarinic cholinergic mechanisms.


Divided attention is a psychological construct that is characterized by assumptions about the fixed finite capacity of subjects to simultaneously process multiple sets of information. While the role of cortical acetylcholine (ACh) in aspects of attention (e.g., vigilance, orienting) is intensively studied, the extent to which cortical cholinergic inputs mediate processing capacity has remained unclear. An operant analogue of the crossmodal divided attention paradigm was developed and the potency of scopolamine to augment speed-accuracy tradeoffs was previously demonstrated (McGaughey, Turchi, Sarter, Psychopharmacol. 115:213-220). In the present experiments, local or global cortical cholinergic deafferentation was achieved by infusions of 192 IgG-saporin into the medial prefrontal or frontoparietal cortex (local deafferentation; Holley et al., Brain Res. 663:277-286) or into the basal forebrain (global deafferentation). Performance following these lesions suggests that local, restricted loss of cortical cholinergic inputs does not robustly affect the animals' ability to divide their attention between the processing of auditory and visual response rules, contrasting with the effects of global deafferentation. Cortical cholinergic mediation of processing capacity may vary mass-action rules rather than being critically dependent on single areas.


The study of the cognitive functions of cortical acetylcholine (ACh) provides experimental approaches aimed at measuring the activity of cortical cholinergic afferents in relation to cognitive activities which recruit this system. Animals were trained in an operant vigilance task (McGaughey et al., Psychopharmacol. 117:345-357) Animals were prepared for the measurement of cortical ACh efflux as described in Moore et al. (Brain Res. 627:267-274) for multiple single unit recording (Givens, Neurosci. press) Medial prefrontal (i.e., prelimbic) ACh efflux increased as a result of transfer of the animals to the test chamber and task onset. Presentation of "background noise" (houselight flashing at 0.5 Hz) during one of the 4 blocks of trials significantly impaired performance, increased cortical ACh efflux and increased spontaneous prelimbic neuronal activity. Different groups of animals were trained in a series of behavioral control procedures assessing the role of motor performance, response habits, reward loss, and sensory stimuli in ACh efflux and neuronal activity. The results from these experiments provide the basis for a discussion about the procedural and conceptual issues important for studies on the cognitive functions of cortical ACh.


Cortical cholinergic deafferentation was produced either by bilateral infusion of 192 IgG-saporin into the basal forebrain (0.42 mg/ml, 0.5 μl/hemisphere) or by infusions of the immunotoxin into the cortex (a total of 5 infusions/hemisphere; 0.01 mg/ml, 0.5 μl/infusion; see Holley et al., Brain Res. 663:277-286). Following surgery, control animals and lesioned animals were retrained in an operant procedure designed to measure the animals’ ability to detect visual signals (presented for 25 - 500 msec) and to discriminate signals from non-signals, i.e., behavioral vigilance. Additionally, the hypothesis that administration of the benzodiazepine receptor partial inverse agonist FG 7142 or the cholinesterase inhibitor physostigmine improves the lesioned animals’ performance was tested. Basal forebrain infusions resulted in > 50 % loss of frontoparietal cortical ACh-positive fibers. Intracortical infusions decreased fiber counts by less than 50 %. Basal forebrain lesions resulted in a persistent impairment in the animals’ ability to detect signals but did not affect their ability to correctly reject non-signal trials. Beneficial drug effects were not found in basal forebrain lesioned animals, possibly because of the almost complete loss of cholinergic neurons. These findings are compared with the data from the intracortically infused animals, and the usefulness of the two models will be discussed.


Immunotoxic lesion provides a more selective model for testing NGF effects on the cholinergic basal forebrain. Rats received i.c.v. PBS or 192 IgG-saporin to produce either 50% (4μg) or 80% (8μg) overall depletions of ChAT activity in four cortical regions, hippocampus and olfactory bulbs. Half of each group was then treated i.c.v. with either 10μg/kg NGF or cytochrome c for 7 weeks. Behavioral testing was performed in treatment weeks 5-7. In each brain region examined, NGF produced the greatest increase in ChAT activity in the lesioned group and the least increase in the highly lesioned group. NGF-treated animals showed a decrease in single-trial passive avoidance retention and an increase in acoustic startle response associated with decreased habituation to startle. No lesion effect was observed in either test. In the water maze, highly lesioned rats had a longer latency to reach a visible platform. There was a significant overall effect of lesion and of NGF treatment, but no interaction. Highly lesioned rats were significantly impaired in finding a hidden platform after learning to escape to a visible platform. NGF treatment significantly mitigated this lesion effect. All groups learned to escape to the hidden platform. This study found that NGF affected passive avoidance retention and acoustic startle independent of the cholinergic lesion. Furthermore, NGF partially improved a performance deficit produced by this lesion in the water maze.
CHOLINE EFFECTS ON MAZE PERFORMANCE ARE MODERATED BY SEX, AGE & ACH Availability. K. Schuler*, I. Francis & N. Ward, Psychology Department, University of Winnipeg, Winnipeg, MB, Canada R3B 2E9

Allometric performance in the Morris water maze is known to vary with sex. Males generally are slightly more efficient at this task than are females. Further, performance is also enhanced by pre- and postnatal choline supplementation. This study examined the allometric performance of 160 Long-Evans rats, 80 of which had been exposed to enhanced choline levels throughout gestation and for 21 days after birth. Equal numbers of choline treated and control male and female rats were tested at four developmental stages: pup, prepuberty, midpuberty and adult. Finally half of the animals in each condition were tested following a scopolamine challenge. Overall, animals exposed to supplemental choline had shorter escape latencies. This enhanced performance was most apparent in male pups and prepubertal females. These differences are related to the structure of the dentate gyrus. Following scopolamine administration, animals previously exposed to choline had longer escape latencies than controls, thus suggesting that as well as dentate gyrus structure differ in these groups.


In this study we have analyzed the subcellular localization of cp2 oxygen, a small GTP-binding protein, whose phosphorylation by PKC has been implicated in associative learning of several species. Cp20 subcellular distribution was studied in purified rat cortical I astrocytes by indirect immunofluorescence using a polyclonal primary antibody affinity purified. Western analysis showed that this antibody specifically reacted with cp20 in the rat astrocytes. Immunofluorescence studies demonstrated that cultured astrocytes exhibited high cp20 immunoreactivity within the nucleus. In addition, optical sections of astrocyte nuclei stained with a confocal microscope confirmed the nuclear localization of cp20 immunoreactivity in the astrocytes. Although cp20 has been shown to be closely related to ARF-class GTP-binding proteins, such as Sar1p, we tested whether small GTP-binding proteins have the same subcellular localization. Surprisingly, no immunoreactivity was observed in the nucleus of astrocytes following immunostaining with an antibody recognizing Sar1 protein, suggesting a peculiar role for cp20 in rat cortical astrocytes. Since cp20 is specifically phosphorylated by PKC, we tested the effects of phorbol esters upon subcellular localization of cp20. In serum-deprived astrocytes treated with 100 nM phorbol myristate acetate (PMA) for a period of time ranging between 15 and 180 min, cp20 nuclear immunoreactivity was no longer detectable from the nuclei of the astrocytes. However, in the presence of serum, time-dependent treatments with PMA did not affect the immunostaining for cp20. Accordingly with our previous observations, these findings suggest that cp20 may play a role in the control of gene transcription, and moreover that PKC may play a dual role of cp20 function in rat cortical astrocytes.


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DEVELOPMENTAL INCREASE IN THE BEHAVIORAL INDUCTION OF FOS PROTEIN IMMUNOREACTIVITY IN AREA CA1 OF THE RAT HIPPOCAMPUS. Nicholas S. Walters*, Anna Y. Kirtikova, and Thomas C. Foster, Department of Psychology, University of Virginia, Charlottesville, VA 22903-0206

Juvanile rats are unable to perform a number of learning and memory tasks which, in adults, appear to require an intact hippocampus. This inability appears despite competent sensory-motor systems, and the ability to perform other learning tasks. Hippocampal responsiveness following exposure to a memory task was studied in juvenile rats, using immunocytochemistry to FOS. FOS protein is the product of the immediate early gene c-fos, and has been considered a marker of cellular activity. Thirty-three littermate pairs served as experimental and control animals, and were tested at P16, P23, and P50 (birth-PT0). Experimental animals were tested for 5 min in a water-motivated reward (water-motivated alternation) or a novel environment for 55 min. Then perfused. Controls were sacrificed immediately after removal from the home cage. Immunocytochemistry for FOS protein was performed on free-floating, 35 m sections of the dorsal hippocampus. Stained sections were digitized using an Olympus CUE-2 densitometry system, and cell counts performed for CA1 regions in the hippocampus and dentate gyrus. There was no effect of age on baseline FOS expression in any region. The behavioral manipulation resulted in a significant increase in FOS immunoreactivity, only in P30 subjects, and this effect was restricted to area CA1. This increase coincided with a significant increase in alternation behavior at the same age. These findings suggest that developmental changes in CA1 are related to the emergence of spatial alternation behavior, and are consistent with previous findings of pre-motor changes in the CA3-CA1 system at this age. This work was supported, in part, by NIH Grants NS318230 to TCF, and HD07323.

STIMULUS NOVELTY EFFECTS NACL TASTE-INDUCED EXPRESSION OF C-FOS IN THE CNS OF GOLDEN HAMSTERS. M. A. Barry*, S. J. Cherkasky, and S. E. Biotechnology, Mt. Sinai School of Medicine, New York, New York 10029-1050

We are using the expression of the immediate early gene, c-fos, as a marker for neuronal activity and protein synthesis induced by gustatory stimulation. We first showed that a series of presentations of 0.1 M sucrose or 0.15 M NaCl resulted in stimulus facilitation in golden hamsters by utilizing the phenomenon of learned helplessness in a conditioned taste aversion (CTA) paradigm. Hamsters with preexposure to the unconditioned stimulus showed a significantly reduced aversion to the novel stimulus following exposure to those preexposed to water. Learned helplessness had not been previously demonstrated in hamsters. For studies of c-fos expression, animals were either preexposed to NaCl (familiar group), or water (novel group). The hamsters were then water deprived and given a dilute of 0.15 M NaCl. After two hours the animals were sacrificed, and their brains were processed to reveal c-fos protein with immunohistochimistry. There was greater c-fos expression in the novel than the familiar group in many (but not all) areas of the brain including gustatory parts of the parabrachial nucleus and insular cortex. Thus a formed memory for familiar stimuli effects c-fos expression associated with gustatory stimuli even in brainstem gustatory nuclei. Supported by 5P01-DH000168


Using immunocytochemical methods we investigated the influence of a visual learning process of the expression of two IEGs, c-fos and zif-268, in the primary visual cortex of the adult cat. We compared the expression levels of both IEGs in the visual cortex and somatosensory cortex (SII) from different cats before and after being trained in a bar orientation discrimination task at different performance levels. In the visual areas 17 and 18, but not in SII, we detected different degrees of c-fos and zif-268 expression depending on the experience of two conditions. The expression level of c-fos and zif-268 was elevated in the bar orientation discrimination cat after five days of training, during which the cat really learns to distinguish between two bars of different orientation. In contrast, the expression levels of both IEGs in the brains, i.e. before orientation discrimination training, or after completion of the training (75% correct for an orientation difference of 5°) in bar orientation discrimination revealed a basal level of c-fos positive nuclei in the supra- and infragranular layers, while the expression of zif-268 immunoreactivity was significantly increased at the very level in layers II, III, IVc and VI of area 17 and 18 in a full-trained cat. Our results show that a discriminative learning process induces c-fos and zif-268 expression in areas 17 and 18 of the cat visual cortex.
EFFECTS OF MATERNAL EXPERIENCE ON FOS-LIR IN MPOA, AMYGDALA AND CORTEX. A.S. Fleming and M. Korenman. Erindale College, University of Toronto, Mississauga, L5L 1C6, Ontario.

In order to determine which brain sites are activated during the acquisition and retention of a maternal experience in postpartum rats, two studies examined the number of cells showing fos-like immunoactivity (Fos-LIR) in brains following re-exposure to pups (USs and CSs) and pup-associated cues (CSs) in maternal-experienced and inexperienced rats. In the first study, day 1 postpartum rats were given a 5 hr exposure to pups and then re-exposed to pups or to a neutral stimulus (performed box) 4 days later. In the second study, day 1 postpartum rats were given a 4 hour interactive experience with pups and a neutral box in a new cage and were then left undisturbed. On day 10 postpartum animals were exposed either to pups in the perforated box in the new cage (USs and CSs), to the box and cage without pups (CSs alone), or left in the home cage. For both studies, at the end of the 10 min exposure the males were sacrificed and brains were prepared for immunohistochemical detection of Fos-LIR.

In both studies the brain site showing the most consistent difference between experienced and inexperienced animals was the medial preoptic area (mPOA), an area of the brain known to be important for the expression of maternal behavior. Fos-LIR was higher in the mPOA of the experienced groups. Marginal experience effects were also found in the bedavolar amygdala, an amygdaloid structure involved in the formation of associations within biologically-relevant contexts. The third site showing experience effects (in the first study only, where animals actually physically interacted with pups on re-exposure) was the parietal cortex. In a fourth site, the prefrontal cortex, experienced animals showed lower Fos-LIR than did inexperienced animals, an effect that may reflect exposure-based habitation to novelty of the pup stimulus.

Supported by MRC Grant to A.S. Fleming.

ANALYSIS OF MEMORY CONSOLIDATION USING INTRACEREBRAL INFUSIONS OF ANTISENSE OLIGONUCLEOTIDES TO THE TRANSCRIPTION FACTOR CREB. G.D. Novak and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Department of Psychology, University of California, Irvine, CA 92717-3500.

The regulatory transcription factor CREB is co-ordinately expressed in the brain and can be phosphorylated by both PKA and CaMK, which are activated by CREB and Ca2+, respectively. Phosphorylation of CREB activates transcription of genes containing AP-1 response element (CREs) in their promoters. Thus, CREB and related transcription factors provide the link between changes in second messenger levels to changes in the animal's transcriptional program. Recent publications have shown a role for CREB in long-term memory consolidation. Thus, CREB and related transcription factors provide the link between changes in second messenger levels to changes in the animal's transcriptional program. To gain a greater understanding of the role of CREB in the consolidation of different tasks in the mammalian brain, we have initiated studies in which antisense CREB oligonucleotides are stereotaxically infused into defined regions of the rat brain and the effect on learning and memory are compared to buffer and mismatch oligonucleotide control groups. The hippocampus and the amygdaloid complex have been chosen for study because of their well documented role in the learning and memory of different tasks. Tasks chosen for study are the hidden platform water maze and continuous multiple trial inhibitory avoidance (CIA). Preliminary results indicate that CREB antisense oligonucleotides infused into the amygdala do not affect memory consolidation in CIA. These results suggest that hippocampal, but not amygdaloid, CREB mediates changes in transcription important for memory consolidation.

Supported by T32 AG00096-12 (FGand USPHS MH51526 (LM)).

GLUCOSE PREFERENCE AND RECOGNITION OF PERIPHERAL GLUCOSE UTILIZATION IN THE BRAIN OF RATS WITH INSULIN-DEPENDENT DIABETES MELLITUS BY A FUNCTIONAL MRI AFTER INSULIN TREATMENT. K.Totsu, T.Yokawa, K.Takuma, Y.Koyama, Y.Kurihara, A.Ogino, I.Tori, Department of Medicine, Tohoku University School of Medicine, Sendai, Japan.

The patients with diabetes mellitus generally display a strong preference for sweetened energy sources to compensate for disorder of peripheral glucose utilization by failure of insulin function. Either preference for glucose or change of the brain function treated with insulin was examined before and after streptozotocin (STZ) administration to Sprague-Dawley male adult rats. Preference for glucose was measured in 10 rats by preferential behavioral tests 2 hours after STZ treatment (50 mg/kg BW, i.p.) and reached a plateau level for a week. Histological findings of the pancreas also supported these behavioral changes as an insulin dependent diabetes mellitus animal. Each animal was then sacrificed and the brain was weighed at 20 min, 30 min, 1 day, 2 week and 4 weeks after STZ treatment. The brain of STZ rats showed preference for glucose 2 weeks after STZ treatment.

Supported by T32 AG0006-12 (FGand USPHS MH51526 (LM)).

673.18

REF-1 PROTEIN EXPRESSION FOLLOWING FEAR CONDITIONING. O. Sheek, S. Milanovic, O. Laban, and J. Splie, Dept. of Molecular Neuroendocrinology, Max-Planck Institute for Experimental Medicine, Hermann-Rein-Slr. 3, D-37075 Goettingen, Germany; 2 Central Institute for Mental Health, JS, D-68159 Mannheim, Germany.

Male mice (C57BL/6J) were subjected to fear conditioning in order to investigate specific changes in the expression of ref-1 protein. Freezing frequency and locomotor activity were evaluated as behavioral parameters. Heart rate was monitored to assess stress levels. In the training phase animals were placed for 3 min into a conditioning box, then presented with a pulsed (5 Hz) tone stimulus (1 kHz, 80 dB, 30 s) before a foot shock (2 s, 0.7 mA) was applied. Animals were tested the day after training either for contextual fear conditioning by placing them into the same box for 6 min without auditory or foot shock stimulation or for tone-dependent retention in an altered context. In addition, unpaired conditioning was performed to determne the retention specificity of ref-1 protein expression.

Ref-1 is a nuclear protein controlling the activity of homo- and heterodimers of immediate early genes (c-fos, c-jun) by exon activation and also functions as a putative/apoptotic DNA repair enzyme. Ref-1 protein could be an important molecular signal in the proposed cascade from ion channel activation to learning and memory.

In brains of control mice, ref-1 protein was highly expressed in the hippocampus (CA1, DG), frontal and perietal cortical areas, and in noc. septalis lateralis and medialis. A time-dependent differential expression of ref-1 protein was observed in the time range from 3-10 min following the training phase with a peak difference at 60 min. At that point ref-1 protein expression was absent in the hippocampus and strongly increased in the 2 and 3 layer of the perietal cortex. After 180 min the ref-1 protein expression pattern was identical with the one in control animals. Differential expression patterns of ref-1 protein following contextual and tone-dependent retention will be discussed.

673.19

CREB LEVELS ARE REDUCED IN THE RAT HIPPOCAMPUS FOLLOWING OVARIECTOMY AND HYPOGLYCEMIA-INDUCED SEIZURES. K. Kojima, K. T. Pikkarainen, Y. Kojima, K. T. Pikkarainen, Y. Kojima, K. T. Pikkarainen. 1Dept. of Pharmacology & Experimental Therapeutics, 2Dept. of Oral Biology, 3Center for Neurobiology of Aging and Stress, 4Dept. of Pharmacology, University of Florida, Gainesville, FL 32610.

cAMP response-element binding protein (CREB) is a transcription factor which has been implicated in the activation of protein synthesis required for long-term memory. Previous research from our laboratory has shown that memory is compromised following hypoglycemia and that some of these memory deficits can be ameliorated with estrogen replacement. We have also reported cognitive deficits following severe hypoglycemic episodes in rats. Given that CREB is critical for memory, we investigated the levels of CREB in rat hippocampus in these two models of memory dysfunction. In Experiment 1, immunoblot analysis of proteins (IIDS-PAGE using polyclonal antibodies to CREB revealed that CREB had decreased by 21% in the hippocampus following one week of ovariectomy while the estrogen-replaced animals showed a recovery in CREB levels compared to ovariectomized animals. This suggests that estrogen treatment may be beneficial in preventing the decrease of CREB in rats, thereby playing a role in memory consolidation. In Experiment 2, we found that insulin-induced seizure reduced CREB by 59% compared to controls in the hippocampus. Immunohistochemistry revealed a decrease number of CREB positive cells in CA1, CA3, dentate gyrus, and the entorhinal cortex. Taken together these results suggest that CREB is an important mediator factor in memory loss following ovariectomy and severe hypoglycemic episodes. (Supported by NIA AG 10485 to JWS).
674.1
DISHABILITATION OF TAIL-Pinch-INDUCED GLUTAMATE AND LACTATE RELEASE BY LOCAL PERFUSION OF RECEPTOR ANTAGONISTS AND EXPRESSION OF PROENKEPHALIN A.

To clarify the roles of rat medial frontal cortex (mPFC) responding to stressful stimuli, we have been studying the habituation of stressful stimuli-induced glutamate (GLU) and lactate (LAC) release by using continuous in vivo brain microdialysis with less than 1 min-resolution. In brief, the dialysate was mixed directly with an assay solution in a T-tube and GLU or LAC was measured in a flow cell under the freely moving condition. We have reported the following. 1) The mPFC GF was increased immediately and transiently from its steady state level after 1min tail pinch (TP). The mPFC GF was also increased by various stimuli (TP, fear, 100dB noise, and 5Hz vibratolization). 2) The 2nd response to the same stimuli given 1 hour later was smaller than the 1st response (about 35% decrease). 3) GF and LR both showed a decreasing response in stress. The mPFC GF response subsided under reseparation while the LR was completely abolished. In addition, we found that the habituation of TP-induced GLU or LAC release disappeared by perfusion of DA (10^(-3)M) or MDMA (10^(-4)M) into the probe for 5min before the two trials in a manner of dis-habituation. These treatments increased the 2nd TP-induced response of GLU or LAC compared with the 1st one (GLU: 153±4±11.4% [n=7], LAC: 360±255% [n=3]). These results suggest that there are at least two different systems for information storage processing specialized in mPFC.

674.2

Proenkephalin (ENK) is expressed in the paraventricular nucleus (PVN) of the hypothalamus where it is regulated by a number of physiological stimuli. Excitatory and inhibitory amino acid neurotransmitters are present at high levels within the PVN and have putative roles in hypothalamic neuroendocrine regulation. Using a hyperosmotic stimulus in rats, we have found that the endogenous expression of a proenkephalin-β-galactosidase fusion gene we have studied the effects of excitatory and inhibitory amino acids on the expression of the proenkephalin gene in the PVN under basal conditions and in response to 150mm saline stress. The GABA-ergic drugs amino- and benzamidine inhibit basal and stress-induced expression of the transgene. MK-801 (a non-competitive NMDA antagonist) inhibits basal expression and 150mm saline stress-induced expression of the transgene. The excitatory amino acids, NMDA and kainate, do not induce proenkephalin gene expression in the PVN after acute or chronic (4 days) administration. Alterations in transgene expression were compared with alterations in Fos expression in the PVN using the same paradigms in vivo and in hypothalamic cultures. A dissociation between transgene and Fos expression was seen following administration of some of these drugs. These results show that excitatory and inhibitory aminoacids can modulate basal and stress induced proenkephalin gene expression in the PVN and may be used to probe the role of negative transcriptional regulation of the proenkephalin gene in this model.
764.7 RESTRAINT STRESS AND ACUTE ETHANOL ADMINISTRATION ALTER ENDGENOUS LEVELS OF A NEUROACTIVE STEROID. D.A. Fins*, A.J. Roberts and J.C. Crabbé. VA Medical Center and Dept. Medical Psychology, Oregon Health Sciences University, Portland, OR 97201.

The GABA-agonist neuroactive steroid 3a-hydroxy-5a-pregnan-20-one (3a,5a-P) can reach endogenous levels which potentiate the effects of GABA in vitro, suggesting that it may be a physiologically relevant neuromodulator. With the single exception of the report that 10 min. swim stress significantly increased brain and plasma 3a,5a-P, there are no available data regarding the influence of stressors on endogenous 3a,5a-P. Therefore, we measured the time course for changes in plasma 3a,5a-P and corticosterone (CORT) following 30 min. restraint stress and ethanol injection (4 g/kg) in genetically heterogeneous male mice. Restrained stress significantly increased plasma 3a,5a-P and CORT, which was evident immediately post-restraint (± 30 min). Plasma 3a,5a-P remained significantly elevated at 60 min. while plasma CORT remained elevated at 60 and 90 min. Both steroids had returned to basal levels at the later time points (150 and 210 min). This result contrasts with the report that swim-stress-induced increase in plasma 3a,5a-P did not occur until 60 min post-stress. Acute administration of ethanol produced a significant increase in plasma CORT at 0.5, 2 and 7 hrs post-injection and a significant decrease in plasma 3a,5a-P at 7 hrs post-injection. Withdrawal severity, measured by an increase in handling-induced convolution scores peaked at 6 hrs and remained elevated at 12 hrs post ethanol. Collectively, these findings suggest that there are differences in the stress response, measured by plasma 3a,5a-P and CORT, depending on whether swim, restraint or alcohol withdrawal was the stressor. Supported by grants NS18964 (DAP), AA18261 (JCC) and the VA (JCC).

764.8 THE EFFECTS OF MICROGROOVING ON GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) AND BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) mRNA EXPRESSION IN THE RAT BRAIN. B. W. Fridell-J. E. Day. Dept. of Biology, The Pennsylvania State University, University.

The objective of this study was to examine the effects of microgrooving on neurodegeneration in rats using in situ hybridization (ISH) and BDNF mRNA expression. BDNF mRNA expression is a useful astrocytic marker that increases in response to injury, neurodegenerative disease, and aging. BDNF plays an important role as a growth factor in several neuronal populations. The rat brain was microgroomed with a Morphogen Factor (BMF) as part of an experiment to test this growth factor's actions on bone density loss in microgravity. Twenty-four over-matured adult female Fisher 344 rats obtained from NASA through the Penn State Center for Cell Research, were housed individually and divided into four groups: 1) vehicle-injected, 2) BMF-injected, 3) vehicle/injected controls, 4) BMF-injected controls. The flight animals were exposed to microgravity (PSEB) for 14 days, and at the conclusion of the flight, brains were removed and frozen in for in situ hybridization. Grain densities per cell were determined on microglia and neurons in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum. Significant differences were found in the pons and cerebellum.
764.13
STRESS-INDUCED INCREASES IN REGIONAL BRAIN CONCENTRATIONS OF NEuropeptide Y-LIKE IMMUNOREACTIVITY.
E. P. Rebec*, R. C. Simard, R. Leduc, J. F. Poullenger, and A. Cadieux. Depts. of Psychiatry and Pharmacology, University of Sherbrooke, Sherbrooke, Que, Canada, J1H 5A1

Experimental evidence suggests an important implication of neuropeptide Y (NPY) in anxiety. For example, we have recently shown that NPY plasma concentrations were significantly higher in panic-disorder patients (Society for Neuroscience abs. 1994). To better understand the relationship between NPY and anxiety, we examined the effects of experimental stress on NPY concentrations in several brain regions in rats. Animals (male Long Evans rats, 250-275g) were separated into two groups (8x): one control and one experimental stress. Animals were handled for 10 min. every day for four days prior to the experiment. On the fifth day, a standard restraint stress was imposed on experimental animals for one hour. Immediately after, dissection of the following regions was performed: nucleus accumbens, striatum, septum, hypothalamus, hippocampus, amygdala, frontal cortex and prefrontal cortex. NPY concentrations were determined by radioimmunoassay. Results revealed that, in comparison to control animals, NPY levels in stressed rats significantly (p<0.05) increased in septum (5.4 pmol/g vs 10.2 pmol/g), hippocampus (3.9 pmol/g vs 6.6 pmol/g), frontal cortex (5.9 pmol/g vs 7.8 pmol/g), prefrontal cortex (5.8 pmol/g vs 10.8 pmol/g) and amygdala (4.5 pmol/g vs 15.4 pmol/g). The marked increase in the amygdala is interesting because of the well known involvement of this limbic region in anxiety. There were no significant differences between control and stressed animals in the other regions studied. The results of the present study support the hypothesis that NPY plays a functional role in anxiety. (Supported by Le Reseau en Santé Mentale du Fonds de la Recherche en Santé du Québec.

764.15


A cholinergic dysfunction has been involved in the neurological mechanisms of stress and depression. In addition, the Forced Swimming Test (FST) has been extensively used as an animal model of depression. In the present study, the distribution of muscarinic cholinergic receptors in the brain of rats subjected to the FST was determined to evaluate the cholinergic hypothesis of depression. Male Sprague-Dawley rats were subjected to the FST for 30 min daily during 15 days. The sham group was placed in the same conditions without swimming. Motor activity was automatically analyzed daily before swimming. Saturation studies were carried out in the presence of 8 increasing [3H]-Quinuclidinyl benzilate ([3H]-QNB) concentrations (0.0625-10 nM) in 0.5M PBS. Regional distribution of cholinergic muscarinic receptors was analyzed by autoradiographic methods, using 1 nM [3H]-QNB. Non-specific binding was determined in the presence of 1nM atropine.

In the FST gravity and ambulatory movements exhibited a significant decrease (34% and 46 % respectively) when the data from 1st and 15th days were compared. [3H]-QNB binding parameters were examined in the caudate-putamen, cortex and hippocampus. The Kd and Bmax values were very similar for both groups in the three analyzed areas, suggesting that neither the affinity of the [3H]-QNB nor the maximal number of receptors were affected by the FST. The distribution of [3H]-QNB binding sites did not show significant differences between both groups in the 30 studied areas. To rule out any cholinergic involvement, further analysis of muscarinic receptor subtypes after FST would be of interest since an alteration of one receptor subtype could be masked by [3H]-QNB binding.

DRUGS OF ABUSE: COCAINE V

765.1

REPEATED ADMINISTRATION OF COCAETHYLENE PRODUCES SENSITIZATION TO ITS Locomotor EFFECTS.

Cocaethylene, a metabolite resulting from combined ethanol and cocaine consumption, is a dopamine receptor blocker and psychotomimetic. That its effects, although similar, may not be identical to those of cocaine is suggested by the finding that, in rats, cocaethylene did not induce sensitization following repeated administration (80 mg/kg i.p.) to induce cross-sensitization to cocaine, while the reverse was true for cocaine (Ellsworth et al., Drug Dev Res 30, 189, 1993). To compare further the behavioral effects of repeated administration of cocaethylene, male C57BL/6 mice (n=5/group) were injected with cocaethylene (0, 10, 20, or 40 mg/kg, i.p.) for 3 consecutive days. On the 4th day, cocaethylene (0, 2.5, 10, 20, or 40 mg/kg, i.p.) was administered to all pretreatment groups. During daily trials, mice were removed from their individual home cages and placed in an observation cage where locomotion was recorded for 30 min before and after injection using an automatic activity monitor. Repeated injection of cocaethylene (10, 20, and 40 mg/kg/day x 3 days) produced apparent shifts of the cocaethylene dose-response function to the left, relative to that obtained in chronic saline treated animals. Cocaeethylene pretreatment also induced an increase in the maximal effect compared to saline pretreatment (maximal beam breaks of 446 ± 53 and 205 ± 45, mean ± SEM, cocaethylene vs saline, respectively). Those results demonstrate that cocaethylene was able to induce sensitization to its locomotor effects and, in this respect, is similar to cocaine which also showed sensitization under the present conditions. Therefore, together with the reported substitution of cocaethylene for cocaine in drug abuse, the finding that cocaethylene, like cocaine, induces sensitization to its locomotor effects, suggests that both drugs share effects not only after acute, but also after chronic administration.

765.2

DIFFERENCES IN OPERANT BEHAVIOR OF RATS RUNNING FOR COCAINE VS COCAETHYLENE.
M. A. Raven*, B. D. Necessary, D. A. Danlick and A. Ettenberg. Behavioral Pharmacology Lab, Department of Psychology, University of California, Santa Barbara, CA 93106

The effects of cocaethylene, a psychoactive metabolite of cocaine, were compared to cocaine using an operant runway self-administration paradigm. Rats were trained to traverse a straight-arm alleyway one trial per day for a single iv injection of cocaine (0.5, 0.75, 1.0 or 2.0 mg/kg). Infrared sensors located throughout the length of the runway were used to record the latency to initiate running (start latency), the time to enter the goal box (run time) as well as the intra-alley behavior of the animals. Although start times remained stable and fast, both drug groups demonstrated some ambivalence (approach-avoidance behavior) about entering the goal box. The incidence of this behavior, however, tended to increase with dose for cocaine and decrease with dose for cocaethylene. It is hypothesized that the strength of the positive relative to negative drug effects may be greater for cocaethylene than for cocaine, especially at higher doses.
COCAYEINE EXPOSURE DURING THE BRAIN GROWTH SPURT INDUCES BRAIN WEIGHT DEFICITS W.-J. Chen*†, R.L. Hernandez and L.R. Wendt. Human Anatomy & Medical Neurobiology, Texas A&M University Health Science Center, College Station, TX 77843-1114

The increased prevalence of alcohol and cocaine abuse among women of reproductive age has received considerable attention since the concurrent use of these two substances results in the exclusive formation of a pharmacological active substance, cocacaine (CE). Presently, it is unclear what, if any, effect CE has on the development of the brain. We have investigated the effects of cocacaine (20 mg/kg, i.p.) daily, from postnatal days (PDs) 4 through 9, on fetal brain weight. Male fetuses were given a liquid diet containing alcohol (8.7%) for 15 days. Each day, two or three fetuses were killed by decapitation, and the same batch of rats was killed at 10 days of age. Cocacaine was determined by gas chromatography-mass spectrometry. Chronic alcohol exposure markedly altered the induction of gene expression by cocaine in caudate putamen and hippocampus. The induction of c-fos expression was significantly reduced in the hippocampus of alcohol-treated animals compared to control animals. Taken together, our data suggest that alcohol exerts a powerful inhibitory effect on cocaine actions, and that cocaine alone produces genomic effects similar to those seen in drug withdrawal. These results indicate that alcohol alters gene expression in a manner that is independent of the presence or absence of cocaine.

This research was supported in part by a Term Faculty Development Award and a Research Foundation Award of the State University of New York at Geneseo. Supported by NIH grant DA07364.


Ethanol is known to induce tolerance and dependence, and the acute reinforcing properties of ethanol appear to be modulated by administration of drugs acting at the dopamine receptor site. Partial dopamine agonists are known to act on the dopamine receptors and may be useful in the treatment of alcohol abuse. We have examined the effects of the dopamine agonist, fenfluramine, which binds to dopamine receptors with high affinity and low intrinsic activity. These drugs act as functional antagonists in conditions of high dopamine tone, and they show an agonistic profile in conditions of dopamine depletion (e.g. denervation). The aim of the present study was to evaluate the effects of acute and chronic ethanol treatment with terguride and SDZ 208-911, two prototype partial dopamine agonists, in non-fluid deprived rats trained to drink alcohol (10% v/v) in a free-choice procedure. Acute treatment with both SDZ 208-911 (0.25, 1.6 mg/kg) and terguride (0.025, 4.0 mg/kg) dose-dependently reduced the reinforcing properties of ethanol as measured by lower intake. Similarly, chronic (10-day) treatment with both SDZ 208-911 or terguride (0.2 mg/kg) induced a significant reduction of ethanol intake. In no case was water intake affected thus ruling out possible nonspecific effects of these drugs on motor performance of consummatory behavior. Therefore, the unique pharmacological profile of partial dopamine agonists supports the hypothesis that these drugs may represent a novel potential therapeutic strategy for normalizing dopamine tone during the various phases of the natural history of drug dependence including alcoholism.

HUMAN COCAINE SELF-ADMINISTRATION: USE OF AN ALTERNATIVE REINFORCEMENT PROCEDURE TO EXAMINE POLYDRUG EFFECTS. John W. Rolls*, Stephen T. Haggins and Angela J. Dearman*, Dept. of Psychiatry, Human Behavioral Pharmacology Laboratory, University of Vermont, Burlington, VT 05401-1619.

A choice paradigm in which volunteers select from cocaine, placebo, alternative reinforcers (money) or eat to respond will be described (Behneagle et al., 1994, Life Sciences). This sensitive procedure demonstrates that cocaine self-administration varies as an orderly function of the magnitude of the alternative reinforcer (i.e., money). Said another way, as the magnitude of alternative reinforcer increases, cocaine self-administration decreases. This relation provides a sensitive baseline to examine whether the effects of pretreatment with other drugs alters the behavioral control exerted by cocaine. Such an assessment is important because cocaine is frequently used in combination with other drugs of dependence. To date we have assessed the effects of ethanol pretreatment and have begun to assess the effects of caffeine pretreatment. Results indicate that both drugs alter preference for cocaine self-administration.

DIFFERENTIAl EXPRESSION OF C-FOS FOLLOWING EXPOSURE TO A COCAINE- OR D-AMPHETAMINE-PAIRED ENVIRONMENT. M.A. Klinic†, C.S. Tham and H.C. Elger. Div. of Neuropharmacology, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3

The attribution of incentive salience to a drug-paired environment is of considerable clinical interest as drug craving can result from the presentation of stimuli previously associated with effects of cocaine and d-amphetamine. In the present study a regional expression of c-fos was examined in the mesolimbic striatum of rats exposed to an environment previously paired with either cocaine (50 mg/kg, i.p. X 10 days) or d-amphetamine (1.5 mg/kg, i.p. X 10 days). In addition to the conditioned motor-stimulant response elicited to animals exposed to the cocaine- or d-amphetamine-paired environment, c-fos expression was significantly increased in the orbitofrontal cortex, nucleus accumbens, caudate putamen, ventromedial hypothalamus, and septum, paraventricular nucleus of the thalamus and amygdala in those animals in which cocaine was previously paired with the environment. In addition, c-fos expression was significantly increased in the piriform cortex, dentate gyrus and amygdala only in animals that were placed in an environment previously paired with d-amphetamine. These results indicate that the pattern of neuronal activation elicited by conditioned stimuli previously paired with d-amphetamine differs somewhat from that produced by a cocaine-paired environment. In contrast, acute administration of cocaine and d-amphetamine produce similar patterns of c-fos expression. Interestingly, although the nucleus accumbens is necessary for the unconditioned locomotor effects of cocaine and d-amphetamine, c-fos expression in this structure was not significantly altered by the contextual conditioned stimulus associated with either drug, thus suggesting that different circuits may be involved in the unconditioned and unconditioned effects of cocaine and d-amphetamine.
PLASMA CONCENTRATIONS OF COCAINE AND BENZYLCHONERGIC FOLLOWING INTRAVENOUS COCAINE ADMINISTRATION. A.E. McMillan, A.E. Lehner, B.M. House and C.P. Machuga. Univ. of Kentucky College of Medicine, Dept. of Pharmacology, College of Pharmacy, THRI and Graduate Center for Toxicology, Lexington, KY 40536.

Following either s.c. or i.p. injection, plasma cocaine concentration is characterized by slow first order kinetics and prolonged elevation. The present study examined the plasma concentrations of cocaine and benzylchonergic (BE) following a single i.v. injection of 0.5, 1.0, 1.5, or 3.0 mg/kg cocaine. Male Sprague-Dawley rats (N=32) were fitted with a subcutaneous vascular access port (MacIntyre et al., 1994) and allowed to recover for at least 3 days. Plasma samples (200 µl) were obtained at 8 time points (30, 60, 90, 120 seconds and 3, 10, 20, 30 minutes) following injection and analyzed by single ion monitoring using GC/MS. Derivatization of BE to the trimethylsilyl (TMS) ester (TMS) was performed prior to analysis and the 241/243 m/e ion pair ratio was interpolated to a 9 concentration (0-10,000 ng/ml) standard curve. Identical SIM procedures were established utilizing the cocaine 182, 198, 272 and 303 m/z pairs and the plasma concentration of cocaine was determined by the interpolation to the 183 peak. Peak plasma concentrations of cocaine occurred at 30 seconds in a dose-dependent manner (3.0±1.0-0.5 mg/kg). In both 0.5 and 1.0 mg/kg cocaine groups, plasma concentrations decreased to <0.05 ng/ml by 10 minutes, yet the 3.0 mg/kg group remained elevated (>500 ng/ml) for longer than 20 minutes. Plasma concentrations of BE reached a plateau in the 0.5 mg/kg (80-90 ng/ml) and the 1.0 mg/kg group (100-170 ng/ml) within 10-20 minutes whereas the concentration of BE in the 3.0 mg/kg group was increasing at 30 minutes (>500 ng/ml). The close parallel of the present data to human cocaine kinetics (Evans et al., 1994) indicates the s.c. vascular access port model of i.v. cocaine administration offers a viable alternative to i.p. or i.c. routes for the study of behavioral modifications and neuropharmacology following cocaine administration. (Supported by DA06638, DA01960, & ES05259)

PRECLINICAL ASSESSMENT OF THE POTENTIAL USE OF METHYLPHENIDATE AS A TREATMENT FOR COCAINE ABUSE. C. McNamara and S. Schnick. Texas A&M University, Dept. Psychology, College Station, TX 77843

Three different self-administration paradigms were used in an assessment of the potential use of methylphenidate as a substitution pharmacotherapy for the treatment of cocaine abuse. In the first, rats were trained to self-administer cocaine and then were treated with methylphenidate (0.5 or 20.0 mg/kg) immediately prior to daily 2 hr sessions. Methylphenidate reduced cocaine-reinforced responding across a wide range of cocaine doses (0.05-2.0 mg/kg) to control levels suggesting that the reinforcing effects of cocaine were completely blocked by pretreatment with methylphenidate. However, methylphenidate did not block self-rewarded instrumental behavior behavior on a model of drug seeking behavior, it dose-dependently reinstated extinguished cocaine-taking. Taken together, these data suggest that methylphenidate may be an effective pharmacotherapy for cocaine addiction. However, the drug has potential for abuse, as indicated by its self-administration and may serve as a cue to reinstate drug-seeking behavior. (Supported by DA06825)


Although molecular mechanisms establishing or maintaining long-term behavioral changes induced by abused drugs are largely unknown, drug-induced alterations in neuronal gene expression could play significant roles in these manifestations of neuronal plasticity. We have used subtracted differential display PCR to identify an initial set of 20 genes characterized by altered expression following psychostimulants or morphine. By cDNA hybridization to mRNAs ranging in size from 0.5 to 12 kb, six display brain-specific expression. Sequence analyses reveal several with homologies to neurotransmitter receptor, intracellular signal transducing factor, and stress responsive enzyme gene families. These and other drug-regulated cDNAs encode candidate genes for involvement in long term CNS changes induced by abused drugs.


Cocaine and amphetamine have both psychostimulant properties. Some of their effects have been related to an enhancement of monoaminergic transmission by blockade of reuptake of dopamine or norepinephrine. Experiments were conducted using an in vitro brain slice preparation from rat somatosensory cortex. Intracellular recordings of cortical neurons were made before, during and after bath application of cocaine or amphetamine (0.1-100µM). EPSPs were evoked by electrical stimulation of the underlying white matter. At low concentration (0.1-1µM), both cocaine and amphetamine produced similar or differential actions on sensory cortical circuits and pyramidal neurons membrane responses were investigated here. These results suggest that different monosynaptic systems may be involved with higher concentrations of amphetamine. Also, cocaine-induced local anesthetic action may play a role in the effects of the drug on cortical circuits. Furthermore, identification of vulnerable cell types may be a necessary step in predicting the impact of these drugs on extended neural processes and expanded growth cones. If these in vitro findings are reproduced in neurons, rG(s), may play a significant role in drug-induced synaptic plasticities.

FACILITATION OF BRAIN STimulation REWARD BY MILDLY PSYCHOACTIVE SUBSTANCES: A QUANTITATIVE COMPARISON WITH PROTOYTIC ADDICTIVE DRUGS. M.A. Bzarz, C.M. Pudlak, & R. Kreek. Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.

Considerable evidence suggests that a compound's effect on brain stimulation reward (BSR) provides a useful assessment of its potential addiction liability. Drugs that enhance BSR are generally highly addictive, and drugs that are not addictive usually fail to enhance BSR. Most investigators consider that a qualitative indicator: BSR facilitation suggests the drug has addictive properties. However, earlier work (M.A. Bzarz, Intraocular self-stimulation as an index of opioid addiction liability, . . . Unpublished M.A. thesis, RPI, 1978) suggested that the most specific aspects of facilitation may be important in distinguishing compounds with high and low addiction liabilities. The present study examined the facilitatory effects of three compounds: the prototypic addictive drug cocaine, the nonaddictive dopaminergic nicotine, and pseudoephedrine. Male, Long-Evan rats with bilateral intrahypothalamic stimulating electrodes were tested using a threshold tracking procedure. Daily 30-min test sessions determined the minimum stimulation frequency necessary to maintain responding at 20 presses. Cocaine hydrochloride (1 to 30 mg/kg, i.p.), pseudoephedrine hydrochloride (3 to 100 mg/kg, l.p.), or nicotine bitartrate (0.125 to 1 mg/kg, s.c.) were injected immediately before threshold tracking. All tests were conducted at 72 hrs. Peak threshold-lowering effects were determined within 180-min test sessions. Cocaine produced robust threshold lowering. Pseudoephedrine also lowered BSR thresholds, but the effect was weaker (i.e., <25% reduction, <25% threshold reduction, respectively). Nicotine produced facilitation quantitatively similar to that seen with pseudoephedrine and distinct from the effect seen following cocaine. These data suggest that quantitative aspects of facilitation must be considered to distinguish compounds with a high addiction liability (e.g., cocaine) from substances with a low addiction liability (e.g., pseudoephedrine). These data further suggest that nicotine's profile in this animal model is that of a nonaddictive substance.
**WEDNESDAY PM**

**DRUGS OF ABUSE: COCAINE V**

**765.15** THE EFFECTS OF MEDIAL FRONTAL CORTEX LESIONS ON COCAINE SELF-ADMINISTRATION AND CONDITIONED COCAINE-SEEKING BEHAVIOUR IN RATS. B. Wassermann*, T.W. Robertson and J.R. Everitt Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, UK.

The medial prefrontal cortex (mPFC) may have an important function in mediating the reinforcing effects of psychostimulant drugs - most likely through its role within cortico-limbic feedback loops connecting the cortex and specific basal forebrain areas with the ventral striatum. The present series of experiments was designed to examine the detailed involvement of the mPFC in different aspects of conditioned and unconditional cocaine-seeking behaviour in rats. Quinolinic acid injected into the mPFC resulted in significantly shorter inter-injection intervals compared to saline controls. The reinstatement of cocaine seeking was associated with increased and maintained levels of i.v. cocaine self-administration, compared to sham-leashed controls (p<0.05). This lesion effect was specific to cocaine-reinforced responding, since both groups extinguished responding when control lever was presented at the same site. Within- and between-session dose-effect curves to cocaine were also determined. Medial PFC lesions produced significant increases in response rates both on the ascending limb and at the top of the descending limb of the inverted U-shaped dose-effect curve, but no shift to the left of the entire function was observed. When responding resulted in saline infusions only, lesioned rats continued to discriminate between active and inactive levers, while control rats responded on both levers at equal rates. Together, these observations suggest the hypothesis that excitotoxic lesions of the mPFC may enhance the preferential expression of a second-order rather than alter cocaine's ability to maintain operant responding. Preliminary data further suggest that mPFC lesions may impair the escalation of cocaine responses under weaker schedules of cocaine reinforcement, indicating a deficit in the extent to which cocaine-seeking behaviour is controlled by drug-associated cues. This work was supported by MRG Grant No.9007194N.

**765.16** OPENFIELD AND PASSIVE AVOIDANCE BEHAVIOR OF YOUNG ADULT RATS PRENATALLY EXPOSED TO ETHANOL, ETHANOL OR BOTH DRUGS OF ABUSE: K. Foley, and C. Libardi, Dept. of VACMP, Washington State University, Pullman WA 99164-6200.

Drugs of abuse such as cocaine and alcohol are known to cause behavioral deficits. When cocaine and alcohol are abused simultaneously, the transsensitization product, cocaineethanol, is formed. Cocaineethanol is more potent than cocaine in some systems, and is known to cross the placenta barrier. Few studies have characterized the effects of cocaineethanol and cocaine on the behavior of some animals. In this study, the effects of prenatal exposure to cocaine, alcohol, or both drugs of abuse on an openfield as well as memory retention in a passive avoidance paradigm were studied. Timed-pregnant Sprague-Dawley dams were treated on gestational days 11-21 with one of the following: (1) free-fed, (2) nutritionally matched to drug-treated dams (pair-fed), and (3) cocaine (20 mg/kg) daily. Low dose of alcohol (EtOH-H2O: 30% of calories as alcohol) 5) high dose of alcohol (EtOH-H2O: 60% of calories as alcohol), 6) EtOH-lo and cocaine, 7) EtOH-h and cocaine. At birth, pups were fostered to surrogate dams to avoid possible maternal potenial effects of the drugs. As adults, rats were tested in a weekly openfield behavior tests followed by training on a standard dark-light passive avoidance paradigm.

In an openfield behavior tests males from the EIOH treatment groups demonstrated significantly reduced margin time as compared to pair-fed controls. These males also showed a significantly greater latency to enter after shock conditioning then females. Alcohol treatment significantly increased the latency to enter in the passive avoidance paradigm. Supported by funds provided for medical and biological research by the State of Washington Initiative Measure No. 171 to CU.

**DRUGS OF ABUSE: COCAINE VI**


Previous research suggests a positive relationship between saccharin preference and the self-administration of ethanol (uric acid) and morphine (J-v) in rats. This experiment was performed to determine whether such a relationship existed between saccharin preference and cocaine self-administration. Male rats (n=32) were given free access to saccharin and water for 4 days. Total daily fluid intake over 4 days was then expressed as a percentage of intake when only water was available. Rats varied in the degree to which saccharin increased total intake, with values ranging from 79 - 237% of water intake. J-vectorial contrasts were then determined at each of the rats. For example: (1) the rats that consumed less than 10% of water intake. (2) rats that consumed more than 10% of water intake and (3) rats that consumed between 10% and 20% of water intake. These conditions, rats from the middle third of the distribution of saccharin preferences self-administered more cocaine than rats with high or low preference, in these conditions, rats showed a higher level of intake (p<0.05). Following the overnight session, all rats were tested in daily 1 hr sessions at doses of 123 - 0 mg/kg and with different preference schedules of FR 1 - FR 6. A significant relationship between saccharin preference and cocaine self-administration was observed only under conditions of a FR 6 schedule combined with a low dose (0.125 mg/kg). Under these conditions, rats from the middle third of the distribution of saccharin preferences self-administered more cocaine than rats in the upper or lower thirds. This "invented U-shaped" relationship between saccharin preference and cocaine self-administration differs from that observed with ethanol and morphine. These results may be related to the observation by Glick et al (Brain Res. 63: 148-154, 1974) of a curvilinear relationship between baseline levels of dopamim in the medial accumbens and cocaine self-administration. Supported by NIDA DA05471, DA06827 and DA06210.

**766.2** PREEXPOSURE TO COCAINE ATTENUATES COCAINE-INDUCED TASTE AVERSIONS. H.F. Diamond* and A.L. Riley. Psychopharmacology Laboratory, Department of Psychology, The American University, Washington, DC 20016.

Animals exposed to one of a number of compounds prior to taste aversion conditioning with those compounds display attenuated aversions relative to nonexposed subjects. In the present experiment, the effect of exposure to cocaine on cocaine-induced aversions was assessed. Cocaine was examined within this preparation because in other designs chronic exposure to cocaine potentiates (or sensitizes) subsequent responsivity to cocaine. In the present experiment rats were preexposed to saline or administered cocaine (32 mg/kg, SC) or vehicle every fourth day for a total of five drug exposures. Animals were then given a novel saccharin solution to drink and immediately injected with cocaine (32 mg/kg, SC) or the distilled water vehicle. Animals preexposed to vehicle and injected with cocaine during conditioning rapidly acquired a taste aversion for cocaine. Animals preexposed to cocaine and injected with cocaine during conditioning failed to acquire a taste aversion, drinking at levels comparable to controls (i.e., subjects preexposed to cocaine but injected with vehicle and subjects preexposed to the vehicle and injected with vehicle). Similar to other drugs within this preparation (both abused and nonabused), cocaine preexposure weakens (and does not potentiate) subsequent cocaine-induced taste aversions.


In prior work (Sobet & Riley, Coll. Prob. Drug Dep., 1994), we have demonstrated that doses of alcohol and cocaine without effect on schedule-controlled behavior markedly suppressed responding when given in combination, an effect consistent with work from a number of other behavioral and physiological preparations in which alcohol has been reported to potentiate the effects of cocaine. In the present experiments, the interaction of these compounds was assessed within the taste aversion preparation, a behavioral index of toxicity. In Experiment 1, different groups of rats were given 2-min access to a novel saccharin solution following an injection of either alcohol (0.56 g/kg, ip), cocaine (25 mg/kg, sc), the distilled water vehicles or alcohol/cocaine combination. Although neither drug alone decreased the vehicle injected rats' taste aversion, animals injected with the combination markedly decreased saccharin consumption, acquiring an aversion to saccharin after only two saccharin-alcohol/cocaine pairings. Interestingly, alcohol did not potentiate cocaine-induced aversions when cocaine was given ip, although the same combination dramatically affected schedule-controlled behaviors (Experiment 2). These data indicate that the interaction of alcohol and cocaine (when administered subcutaneously) extends to indices of toxicity.


Cocaine abuse is a major public health problem. Cocaine addicts frequently present with psychiatric as well as neuroendocrine abnormalities. Cocaine's influence on the neuroendocrine system has been investigated. Primary research efforts have been directed towards elucidating the hypothalamic mechanisms of cocaine's action. To date however, no studies addressed direct effects of cocaine on the pituitary cells. We have selected a well-characterized model of anterior pituitary corticotrophs, the AtT-20 cell line, to elucidate the direct effects of cocaine on a single component of the neuroendocrine system. Whole cell recordings utilizing current and voltage clamp techniques employed. In addition, intracellular calcium was measured by means of Fura-2 imaging techniques. Images of both cell populations and single cell recordings were acquired. Application of cocaine (300 nM - 3 mM) resulted in inhibition of spontaneous firing. This inhibition was associated with significantly increased outward and to lesser extent inward current. Results from whole cell calcium recordings indicated cocaine induced increase in the resting calcium levels. Single cell recordings support the population studies. The results of this study are in a complex model of cocaine actions on the pituitary corticotrophs and its possible role in the modulation of the excitation secretion process.

Preclinical evidence indicates that chronic cocaine exposure alters dopamine (DA) neurotransmission. In the present study, we examined the effects of repeated intranasal cocaine administration on DA function in human subjects in an in-patient research study. Male polydrug abusers (N = 11) participated in 2 daily self-administration sessions (AM & PM) for 5 consecutive days. Subjects sought both placebo and cocaine (0.1 mg/kg each day), with cocaine being presented in a randomized, double-blind fashion at 1 of the daily sessions. Neuroendocrine challenge tests were performed using the DA receptor agonist pergolide (0.1 mg, p.o.) 5 days before and after the repeated cocaine dosing regimen. Repeated blood samples were drawn at 30 min intervals from 1 hr before until 6 hr after pergolide challenge. Plasma prolactin (PRL) and growth hormone (GH) concentrations were determined byRIA. Pergolide decreased circulating PRL and increased GH. No significant differences in responsiveness to pergolide were found between the pre- and post-cocaine condition. Our data suggest that repeated doses of intranasal cocaine do not modify DA receptor sensitivity in human drug users.


Gravid Sprague-Dawley rats (250-275g) received chronic cocaine infusions throughout gestation; s.c. injections b.i.d. of saline (Sal) or 15 mg/kg of cocaine HCL or 1.5 mg/kg anfeline acid (AFA) once daily. Females were tested on postnatal day 21 for maternal aggression towards a male intruder during a 10 min. period. Cocaine treated dams threatened intruders more than AFA dams (p < 0.05) or Sal (p < 0.04) treated dams. Dams were sacrifice for oxytocin testing using the whole ventral tegmental area and amygdala. Mean picograms per area and per mg protein were compared for the groups. Chronic cocaine treatment significantly reduced oxytocin levels in the amygdala as compared to Sal-treated (p < 0.05) and AFA-treated dams (p < 0.01). (Supported by NIH grant DA08456 and a UNC Medical Faculty Grant).

INCREASED COCAINE SELF-ADMINISTRATION AFTER SOCIAL STRESS: K. A. Miklovic*, N. Hubbard and I. Caminiti-Caravaggio. Dept. of Pharmacology, Taffl Univ, Miami Dade 33155. Cocaine and many kinds of stress lead to increased release of mesocorticinolimbic dopamine. How specific is the activating effect of social stress to cocaine self-administration? We assessed the impact of social stress on behavior that was reinforced either by i.v. cocaine or by food, each at similar rates. Social stress was engendered by exposing the experimental rat solely to the threats of an aggressive resident rat behind a protective screen without potentially injurious attacks. Initially, we determined the characteristic inverted U-shaped concentration-response rate curve for cocaine self-administration (0.016-0.25 mg/mg/in)fusion), maintained by an FR 10 schedule. Each rat was assessed for maintained responding in a parallel group, the other group being a food-reinforced behavior, maintained by a joint FR 10 FI 3 min schedule, was determined before, during, and after daily exposure to social stress. This resulted in a 10 min. habituation to the stressor, and in a second phase, animals were exposed to social stress for 60 min immediately before the daily session, and the cocaine concentration-response rate curve was re-determined. In a parallel group, the other group being food-reinforced responding, was increased in rats that were socially stimulated prior to the daily session. Other stressors like pair housing or exposure to novel environments did not affect cocaine self-administration. This increase was largely due to a high rate of responding immediately after the cocaine infusion pointing to activation of the reward system in animals whose mesocorticinolimbic dopamine is already released due to social stress.

INFLUENCE OF CORTICOTERONE ON THE PSYCHOMOTOR EFFECTS OF COCAINE: A DOSE-RESPONSE STUDY. M. Marinelli, P. Rouquette-Pey, M. Le Mail, and P. V. Passec INSERM U 296, University of Bordeaux I, 33701 Bordeaux, France.

Adrenocorticotropism (ACT) is known to reduce the behavioral effects of cocaine whereas replacement of corticosterone in the range of basal diurnal levels reverses these effects. This study further investigated the relationship between corticosterone (CORT) and sensitivity to psychostimulants. For this purpose, in a first experiment we studied the effects of AIX on the locomotor responses to different doses of cocaine (0, 3.25, 7.5, 15, 30, 60 mg/kg). Locomotor activity increased with the increase in drug concentration reaching the maximal locomotor activation at the dose of 30 mg/kg in all animals, however, this dose was reduced by 60% by removal of circulating of CORT. Thus, AIX rats did not differ from controls either for the response to a saline injection or for the psychomotor activation induced by the lower doses of cocaine (3.125 and 7.5 mg/kg), but at all the higher doses AIX rats showed a lower locomotor response than sham rats. In a second experiment we investigated the dose-response effects of replacing corticosterone on the locomotor response to cocaine (20 mg/kg). In this study AIX animals were implanted with subcutaneous pellets containing different concentrations of the hormone (0, 3.125, 12.5 or 50 mg) for as to progressively increase circulating CORT to levels observed in diurnal conditions. Again, the absence of CORT reduced the locomotor response to cocaine and there was a progressive increase in drug effect with increasing doses of CORT. The effects of AIX were completely abolished by the higher dose of CORT which gave diurnal levels of the hormone. The differences observed in response to cocaine cannot be explained by a difference in drug availability since neither AIX nor CORT replacement had any effect on brain levels of cocaine. These results indicate that stimulant effects of cocaine depend on CORT concentrations and that this hormone facilitates the psychomotor properties of this drug. This study suggests that individual differences observed in sensitivity to drugs of abuse may depend on circulating CORT levels and may open new therapeutic strategies for drug addiction.

NEUROENDOCRINE AND BEHAVIORAL RESPONSES TO COCAINE IN FISCHER AND LEWIS RATS M. Renee Sim* and Nick G. Goodman, Dept. of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71132.

Differences in the vulnerability to self-administer drugs of abuse have been observed in laboratory rats. Reports suggest that differences in adrenocortical activity may be a factor in the variability to drug self-administration. These studies compared the chronic administration of pergolide and behavioral responses in strains of rats previously shown to self-administer drugs differently. Lewis (LEW) and Fischer (F344) rats were chosen for their patterns of self-administration as well as for differences in the responsiveness of the hypothalamic-pituitary-adrenocortical (HPA) axis to various stimuli. The acute pergolide responses to cocaine were characterized by the effect of cocaine (1-60 mg/kg, i.p.) on hypothalamic corticotropin-releasing factor (CRF), corticosterone (CS), and adrenocorticotropin hormone (ACTH). Although both LEW and F344 rats displayed a decrease in brain CRF and an increase in plasma CS and ACTH, the percent response in LEW rats was greater than in F344 rats. This suggests that acute injections of cocaine can stimulate HPA axis activity, and that LEW rats, the drug-prefering strain compared to F344 rats, are more sensitive to the neuroendocrine activating effects of cocaine. Studies on the reinforcing effects of cocaine using an operant model of intravenous (i.v.) administration have also been performed. Rats were tested for acquisition of cocaine self-administration at increasing doses (0.01, 0.032, 0.125, 0.5, and 1.0 mg/kg/infusion). Our data indicate that LEW rats will self-administer i.v. cocaine more readily than F344 rats. Although basal plasma CS is higher in F344 rats, a greater CS response to i.v. cocaine was observed in the LEW strain. These studies suggest that neuroendocrine and behavioral responses to cocaine are markedly different in LEW and F344 rats, and these differences may be a factor in the variability in drug preference observed in these strains. This work was supported by USPHS Grant DA 06019.


Previous stress may change vulnerability to drugs of abuse, however neither the neural mechanisms underlying such effects nor the nature of the stress are well understood. The objective was to study whether social stress, either one week or immediately before administration of morphine or cocaine can influence c-fos expression in brain stem of mice. "Social stress" was defined as defeat by an aggressive resident mouse and subsequent exposure to the resident's threats behind a protective screen. Mice were injected i.p. with morphine (7.5 mg/kg) or cocaine (40 mg/kg) and killed one hour after injections. The non-opioid innervation of the brain was studied by immunoreactivity protein was investigated in periaqueductal gray (PAG) and locus coeruleus (LC). Social (defeat) stress in naive mice increased Fos-positive cells in PAG and LC in comparison to saline or untreated mice. Administration of morphine enhanced c-fos expression in PAG and LC. Social stress immediately before morphine injection appeared to have additive effects on c-fos expression in PAG and LC, whereas social stress one week before morphine challenge attenuated c-fos expression in PAG. Cocaine produced large c-fos expression 1 hour after injection in PAG and LC. A single defeat immediately after cocaine significantly attenuated c-fos expression in PAG and LC, whereas social stress 1 week before cocaine administration diminished c-fos expression in LC, resulting in ca. 50% lower count than after acute cocaine. These results suggest that development of stress-induced tolerance or sensitization to morphine or cocaine, respectively, is related with c-fos expression in brain stem.
WEDNESDAY PM DRUGS OF ABUSE: COCAINE VI

VARIABLE HEMODYNAMIC RESPONSIVENESS TO BEHAVIORAL STRESS AND COCAINE IS RELATED TO DIFFERENTIAL CNS RESPONSIVITY

We have noted highly variable hemodynamic responsiveness to cocaine and air jet stress in a previous publication (AP, 265: H779, 1993). In the present study, we examined the relationship between cardiovascular responsiveness to cocaine and air jet with release of dopamine (DA) and norepinephrine (NE) in the nucleus accumbens (NA) and striatum (Str) as measured by HPLC. Concentrations of DA and NE were determined for both control and cocaine-treated rats. Cocaine and air-jet stress concentrations were highest in the NA and Str, respectively.

In the present study, we examined the relationship between cardiovascular responsiveness to cocaine and air jet with release of dopamine (DA) and norepinephrine (NE) in the nucleus accumbens (NA) and striatum (Str) as measured by HPLC. Concentrations of DA and NE were determined for both control and cocaine-treated rats. Cocaine and air-jet stress concentrations were highest in the NA and Str, respectively.

**DRUGS OF ABUSE: COCAINE VI**

**766.11**

**VARIABLE HEMODYNAMIC RESPONSIVENESS TO BEHAVIORAL STRESS AND COCAINE IS RELATED TO DIFFERENTIAL CNS RESPONSIVITY**

**766.12**

**DIFFERENTIAL EFFECTS OF ACUTE RESTRAINT STRESS ON THE BEHAVIORAL EFFECTS OF COCAINE. J.R. Acti and J.M. Wilkin**

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Acute laboratory stressors result in a number of neurochemical and behavioral effects. Stress has been reported to increase dopamine levels in brain regions associated with subjective and reinforcing effects of abused drugs and has been reported to produce cross sensitization to psychomotor stimulants. The present experiments were undertaken to determine if acute restraint stress could modify the discriminative stimuli effects of locomotor stimulatory effects of cocaine. Six rats trained to discriminate 10 mg/kg of cocaine were restrained for 15-20 minutes, and the dose-effect curve of cocaine was significantly shifted to the left, suggesting a potentiation of subjective effects. Subsequent re-establishment of the dose-effect curve indicated that the stress effect was transient, by a return to baseline values. A second series of experiments evaluated the effects of 15-20 minutes of restraint stress on the locomotor stimulatory effects of cocaine in mice. Naive mice were either restrained for 15-20 minutes or remained in home cages prior to administration of saline or one of several doses of cocaine. Stress significantly decreased locomotor activity, measured as horizontal activity or number of movements, and significantly reduced cocaine-induced increases. When this experiment was repeated with mice that had been previously habituated to the locomotor arena, stress reduced locomotor activity in saline- treated, but not in cocaine-treated mice. These results suggest that stress effects on cocaine-mediated behaviors are complex, dependent upon the behaviors being measured, and can be further modulated by behavioral history.

**766.13**

**CHRONIC EXPOSURE TO CADMIUM ALTERS THE INITIATION OF BEHAVIORAL SENSITIZATION TO COCAINE. J.R. Nation*, C.L. Livermore, and G.R. Bratton**

Texas A&M University, College Station, TX 77843

Cadmium selectively acquires in tobacco plants and poses a significant health hazard to heavy users of tobacco products. Recent findings indicate that risks may extend to include altered sensitivity to drugs that might impact cognitive function. In this line of research, the present investigation examined the effects of recurrent cadmium exposure on behavioral sensitization to cocaine. While monitoring motor activity, control and cadmium-treated adult male rats (60 days exposure to 100 ppm cadmium chloride via water supply), received the daily IP injections of 10 mg/kg cocaine HCl or saline for 14 successive days. On Day 15 all rats received saline injections, followed on Day 16 with 18 incremental challenges of 10, 20, and 40 mg/kg cocaine. The findings showed that cadmium exposure retarded the initiation of sensitization. These data suggest that xenobiotic contamination may alter drug responsiveness and therein may influence patterns of drug selection and use.

**766.14**

**NEUROBIOLOGICAL RELATIONSHIP BETWEEN VULNERABILITY TO DEPRESSION AND TO DRUG ABUSE, IN Vivo MICRODIALYSIS STUDIES. Marino Lopez* and Elliott L. Gardner, Department of Psychiatry, Albert Einstein College of Medicine, 1700 Morris Park Avenue, Bronx, NY 10461-6022.

Clinical studies have shown a marked association between co-occurrence of depression and drug abuse. In agreement with theories that depression is reflected in a reduced capacity to experience pleasure, animal experiments demonstrate that manipulations that predispose towards depression also influence reward processes. Changes in neurotransmission in the mesolimbic dopaminergic (DA) system may underlie this effect. Stress has been shown to increase DA turnover in this system. A mild, stressor can induce abnormalities in DA transmission specific to the nucleus accumbens (NA). The following experiment examined the relationship between the co-vulnerability to depression and to drug abuse. The effect of cocaine on the development of learned helplessness (LH) was studied in animals genetically susceptible (Lewis), non-susceptible (Fischer 344) in drug abuse. Following exposure to LH, animals were implanted with dialysis probes in the NA and, the effect of cocaine challenge (10 mg/kg) on DA transmission was examined. Although, L rats were more susceptible to drug abuse than F344, neither strain demonstrated any predisposition towards LH. When cocaine was administered prior to LH training, 49% of L and 70% of F344 rats became non-LH. Cocaine, therefore, had a protective effect on the development of LH, and was more pronounced in F344 rats. Following cocaine challenge, dialysate DA levels were significantly reduced in both strains. Controls, saline DA was higher in L rats than F344. Following LH training, saline DA levels did not increase significantly in F344 rats compared to controls. However, F344 rats that received cocaine during LH training (and who later became non-LH) showed a greater than 100% increase in dialysate DA levels. The effects of cocaine on dialysate DA in the L rat are currently under investigation. Thus, when cocaine is administered during LH training, the effect on dialysate DA levels in F344 rats is dramatic, and suggests that a potentially significant and long-lasting change in the mesolimbic DA system following cocaine stress could reverse a genetic neo-susceptibility to drug abuse.

**766.15**


Activation of the hypothalmo-pituitary-adrenal (HPA) axis has been implicated in the development of psychosomatic and stress-induced behavioral sensitivity. In an attempt to further characterize the role of the HPA axis in stress-sensitization, the effects of adrenalectomy (ADX) on the initiation and expression of cocaine-induced behavioral sensitization and stress-induced cross sensitization were examined. In order to determine the role of HPA axis in the initiation of behavioral sensitization, male Sprague-Dawley rats were subjected to sham surgery or ADX. A week later, rats were subjected to a sensitization paradigm with unilateral intraperitoneal injections of cocaine (Day 1: 15 mg/kg; Day 2 to 6: 30 mg/kg). On day 7 (early withdrawal) the behavioral response to cocaine challenge (15 mg/kg) was tested. These experiments were repeated with animals under stress sensitization. Stressors involved acute restraint stress and corticosterone in the nighttime drinking water to mimic the circadian variation of hormone levels. After one week, rats were given a saline challenge followed by a cocaine challenge (15 mg/kg) the next day (late withdrawal). Sham controls demonstrated a sensitized locomotor response to the cocaine challenge at both early and late withdrawal times compared to the response on day 1. In contrast, sensitization was completely blocked in ADX rats at both withdrawal times but not at late withdrawal. The effect of ADX on the expression phase of sensitization was examined by administering daily cocaine as before followed by surgery one or two days later. No effect of adrenalectomy was found on the expression of behavioral sensitization. These results suggest that corticosterone is necessary during the initiation of behavioral sensitization of sensitized response at the early withdrawal time but not at late withdrawal time. Similar experiments were done to examine the effect of adrenalectomy on stress-induced cross sensitization. A stress paradigm that included mild foot shock stress and restraint stress was used in place of daily cocaine injections. Preliminary results from these experiments suggest that ADX had similar effects on both cocaine- and stress-induced sensitization.

**766.16**


Different studies show that cocaine administration and cocaine withdrawal produce anxiogenic states in animals. This study was done to examine the behavior of rats in the elevated plus-maze during acute and prolonged cocaine self-administration and after cocaine withdrawal. Adult male rats were allowed to administer cocaine solutions (0.1 - 0.5 mg/kg) using the two-bottle choice technique during different periods of time (30 or 60 days). The amount of cocaine self-administered was in the range from 10 to 30 mg/kg. The animals were trained in the elevated plus-maze during the first 15th and 30th day of cocaine administration and for 5 days after withdrawal. Etiological evaluation of frequency and duration of escape to open and closed arms was further conducted using videorecordings. Behavioral effects were not detected after acute oral self-administration of low doses of cocaine. Decreased entries into the open arms was seen on the third day of treatment withdrawal. Increased entries to the open arms was verified after acute self-administration of higher doses of cocaine. These animals also showed this behavior during the first days of testing after cocaine withdrawal. Financial support: CNRP and FAPERGS.
Cocaine is a powerful drug, with high abuse potential in humans. While considerable progress has been made in understanding cocaine's mechanisms of action using in vitro and animal models, very limited information is available on the temporal and spatial localization of action of cocaine in the human brain. Functional MRI is a non-invasive imaging technique that permits the visualization of functional brain activity with excellent temporal and spatial resolution without the use of ionizing radiation. Single shot echo planar images was obtained on a 1.5 Tesla GE Signa scanner using an insertable, balanced sequence, 3-axial head gradient coil for rapid imaging. Images were acquired through the entire brain volume, a shielded quadrature ellipsoidal endcaps transmit/receive birdcage radio frequency coil was used. Four experienced crack/cocaine users received doses of cocaine (0.3, 1.0 and 40 mg over 60 sec) while fMR data were acquired (TR=6 sec; TE=40 msec; 8 mm slice thickness). Subjects all displayed consistent increases in their received doses and positive subjective effects to the drug. Heterogenous activation patterns included consistent cerebral activation in the bilateral dorsolateral frontal cortex, anterior cingulate, temporal cortex, and left insula. These results were evident in several frontal fields. Signal activation latency generally varied between 1-2 min with duration of effect between 8-20 min depending upon region. These data demonstrate the ability of fMRI to map drug effects in the human brain. (Support by USPHS grant DA09465)
RESPONSE TO NOVELTY PREDICTS COCAINE SELF-ADMINISTRATION IN RATS. J. W. Grimm* and R. E. Seck. Department of Psychology, Washington State University, Pullman, WA 99164-8822.

Rats displaying a higher locomotor response to novelty than rats with a lower response have been shown to more readily acquire amphetamine self-administration. The present study sought to establish that response to novelty predicts cocaine self-administration as well. Subjects were female Sprague Dawley rats (N=10) weighing 220-280g. Response to novelty was recorded as the locomotor response of a rat on a photobeam activity chamber. The animals were then randomly assigned to one of two conditions: novelty acquisition (NA) or novelty extinction (NE). This protocol was designed to establish a baseline of locomotor responses to novelty, which were observed for 4 days prior to drug administration. Cocaine hydrochloride injections were given at a dose of 10mg/kg i.v. daily for 14 days. The animals were then retested for response to novelty, which was then increased by 10mg/kg. This protocol was repeated for the next 14 days. Results indicate that rats that responded more to novelty also showed a higher response to cocaine. This suggests that the ability to respond to novelty may be a predictor of cocaine self-administration.

INDIVIDUAL DIFFERENCES IN COCAINE SELF-ADMINISTRATION: DOSE-RESPONSE AND RATIO-RESPONSE STUDY. V. Dresner, F. Poupe-Pont, M. Le Moal and J.Y. Piazza. INSERM U299, Unv. de Bordeaux II, 33075 Bordeaux, France.

There are considerable individual differences among humans in drug taking behavior. Individual differences in the propensity to develop drug intake have also been evidenced in rats. When intravenous self-administration is studied with a low dose of psychostimulants, all rats will administer the drug during the first day of testing. However, if the psychostimulants are administered in one session followed by a renewal phase, some rats will maintain their self-administration and others do not. In some individuals, the behavior rapidly extinguishes whereas in others it is maintained in the order and can be predicted by the reactivity to stressful situations, and in some individuals a high locomotor response to novelty (independent of drug) predicts self-administration whereas in others a low response to novelty predicts extinction. In this study, we assessed individual differences in cocaine self-administration, and found that the dose of drug (0.05, 0.25, 0.52, 0.69, 2.16mg/kg) was a function of the ratio required to obtain an injection (1, 2, 3, 6, 10). The most sensitive tests of self-administration response were performed by rats that were trained to self-administer the higher doses (7 days). The results suggest that individual differences in cocaine self-administration may be predictive of response to other drugs as well.

A COMPARISON OF THE EFFECTS OF CONTINUOUS COCAINE INFUSIONS ON LOCOMOTOR ACTIVITY AND ON BRAIN STIMULATION REWARD. C.M. Purd*., R. Kukl, & M.A. Rosatti. Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.

The effects of continuous cocaine self-administration on two behaviors—locomotor activity (LMA) and brain stimulation reward (BSR)—were examined. The rats were trained to self-administer cocaine at a rate of 1.2 mg/kg per day. The LMA and BSR procedures were performed separately. The LMA procedure consisted of daily 15-min sessions in which the rats were exposed to a series of escalating drug infusions. The BSR procedure consisted of daily 15-min sessions in which the rats were exposed to a fixed-ratio schedule of cocaine injections. The results indicated that the rats exhibited a marked decrease in LMA and a significant increase in BSR during the study.

COCAINE SELF-ADMINISTRATION ON SECOND-ORDER SCHEDULES OF REINFORCEMENT IN RATS. R. Banuelos*, R. A. Wise and D. C. Roberts. Dept. of Psychology, Ottawa, Canada.

Responding rates were determined for cocaine self-administration under a fixed-ratio schedule of reinforcement (FR-S). The rats were trained to self-administer cocaine at a rate of 1.2 mg/kg per day. The results indicated that the rats exhibited a marked decrease in LMA and a significant increase in BSR during the study.
T67.14 COCAINE-INDUCED PLACE PREFERENCES & AVERSIONS: EVIDENCE FOR AN OPPONENT-PROCESS MECHANISM OF ACTION. A. Eckenberg, M. Raven, D. Danlick and B. Necessary. Behavioral Pharmacology Laboratory, Department of Psychology, University of California, Santa Barbara, CA 93106

Rats running an alley for IV cocaine have been reported to show a unique approach-avoidance behavior with respect to the goalbox. This ambiguity occurs even though the rats initially exit goalbox trials normally, operantly self-administer the same dose of cocaine that they receive in the goalbox, and demonstrate place preferences for environments paired with the same dose of cocaine. To account for these paradoxical results, an opponent-process model was hypothesized in which an initial positive response to cocaine was thought to diminish in strength followed by, or concurrently with, an insurgence of an negative affective state. The present experiment tested this notion using the Conditioned Place Test in which the effects of a 0.75 mg/kg IV injection of cocaine was paired with a distinctive environment for 5 min either immediately post-injection, after a 5 min delay, or after a 15 min delay. Reliable preferences were demonstrated for the cocaine-paired environment in the no-delay condition, weaker preferences were observed in the 5 min delay condition, and significant delay condition. These results support the notion of an opponent-process mechanism of action for IV cocaine.

T67.15 DO CALCIUM CHANNELS IN THE A10 DOPAMINE REGION PLAY A ROLE IN THE DEVELOPMENT OF COCAINE-INDUCED BEHAVIORAL SENSITIZATION? J.D. Steketee* and B.R. Brown. Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Previous studies have suggested that L-type Ca2+ channels may be involved in the development of psychostimulant-induced behavioral sensitization. In particular, pretreatment with the dihydropyridine Ca2+ channel blockers, such as nifedipine or nimodipine, blocked the development of behavioral sensitization. The mesolimbic dopamine system has been proposed to play a critical role in the development of sensitization. The mesolimbic system arises from dopamine cell bodies located in the ventral tegmental area (A10 region) to innervate numerous limbic structures, including the amygdala. The A10 region is thought to be involved in the initiation of sensitization. Thus, in these studies we examined the role of L-type Ca2+ channels in the A10 region on the development of sensitization to cocaine. In one set of studies, animals received daily intra-A10 pretreatment with nimodipine (10 or 30 nmol/d) or saline (0.6 μl/d), a Ca2+ channel blocker, 5 min before a peripheral cocaine injection (15 mg/kg, ip). In a second set of studies, animals received daily intra-A10 injections of Bay K 8644 (10 or 30 nmol/μl) or saline into the A10 region. In both of these experiments, animals received a challenge injection of cocaine 1 week after the last of daily injections. Motor activity was maintained for 2 hr following the first daily injection and on the day of the cocaine challenge. Bay K 8644 did not affect motor activity, nor did daily injections induce behavioral sensitization to cocaine. Nimodipine did not block the acute, nor the development of the sensitized, responses to cocaine. These data suggest that the dihydropyridine sensitive Ca2+ channels in the A10 region do not play a role in the development of cocaine-induced behavioral sensitization.

This work was supported by grants from the Louisiana Educational Quality Support Fund (RD-A-18) and the National Institute on Drug Abuse (DA08709).


Environmental cues previously associated with cocaine abuse have been shown to elicit drug craving and the resumption of drug use in abstinent individuals. Despite the importance of drug associated cues in relapse, few attempts have been made to model this behavior in animals. In the present study, rats exposed to noncontingent presentation of drug paired stimuli following 14 daily hr. daily cocaine self-administration sessions (0.3 mg/saline), and 20 days of 3 hr. extinction trials showed significant reinstatement of responding. Rats placed back in the experimental chamber following 4 days of drug-associated trials and exposure to noncontingent presentation of drug paired cues showed higher responding than animals placed back in the chamber under extinction conditions. In addition, bilateral lesions of the basolateral amygdala (BLA), via N-Methyl-D-Aspartic acid (NMDA) microinjection (0.2 mg in 500 ul vehicle), either in the middle or of following chronic cocaine self-administration increased the rate of extinction and attenuated the ability of the paired stimuli to reinstate responding. Lesions of the BLA following 7 days of cocaine self-administration failed to alter subsequent responding for cocaine. These results are consistent with the hypothesis that the BLA is important for the association of reinforcing with neutral stimuli, but not the reinforcing effects of cocaine itself. Furthermore, this paradigm represents a model for investigating the role of conditioned stimuli in drug craving and relapse.

T67.18 MODULATION OF mRNAs FOLLOWING WITHDRAWAL FROM COCAINE AS REVEALED BY DIFFERENTIAL DISPLAY. D.J. Emiulst* and B.M. Cohen. McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178.

We are currently using differential display reverse transcripase PCR (DDRT-PCR) to identify genes which are regulated following withdrawal from multiple doses of cocaine. Initially the ability of DDRT-PCR to accurately and consistently reveal the regulation of known genes was investigated. RNA samples were isolated from the striatum of rats that had received repeated IV injections of either vehicle (0.9% saline, pH 7.0) or 6 mg/kg cocaine-HCl and subjected to DDRT-PCR with primer pair 9G which is designed to amplify a 240 bp segment (R2420) of cDNA derived from c-fos mRNA. Independent DDRT-PCR reactions consistently indicate that the relative amount of R2420 accurately reflects the level of expression of c-fos mRNA that was previously measured by Northern blot analysis [Emiulst et al., 1994], Mol. Brain. Res. 26:106-112]. Here DDRT-PCR analysis of known genes has the advantage of providing an internal control to confirm the validity of the DDRT-PCR reactions. An anonymous PCR product (R6168) was recently observed reverse transcribed and amplified following the last cocaine treatment, but not at 1 hr. Two other PCR products (R6132 and R6127) are reproducibly transcribed in cocaine treated animals both 1 hr and 1 day after the last exposure to cocaine. Further characterization of these PCR products is ongoing and additional screening with other selected primer pairs is expected to reveal additional candidate genes. This work is supported by grants from NIH and NARSAD.
EPILEPSY: ANIMAL MODELS III

768.1
SEIZURE SUSCEPTIBILITY IN AN ANIMAL MODEL OF NEURAL MIGRATION DISORDERS, S.C. Baraban* and P.A. Schwartzkroin, Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

We investigated the effect of prenatal (E15) methylxanthinocochetal acetate (MAMac), a transgenic agent, on seizures susceptability of the offspring. Creyl-violet-stained sections of hippocampus confirmed the presence of ectopic pyramidal cells in a rat and a strain of mice CA1/CA2. In awake, freely-moving animals (P60) from MAM-acjected dams, the latencies to kindling-induced seizure activity (myoclonic jerk: 173 ± 2.3 s; forelimb clonus: 215 ± 4.9 s) were significantly shorter than those of age-, sex- and strain-matched controls (200 ± 6.9 s and 218 ± 18.8 s, respectively). Shorter latencies were associated with larger numbers of ectopic pyramidal cells. In vitro intracellular recordings from CA1 pyramidal cells in MAM-aciated tissue (P25-P35) were similar in many of their intrinsic properties (e.g. RMP, AP amplitude, Rmp) to cells from control tissue. The synaptic responses of CA1 cells in MAM-aciated and control hippocampus were also comparable. However, MAM-aciated tissue could be distinguished as having an unusually high proportion of CA1 cells (>60%) which fired a burst of action potentials in response to subthreshold current injection. Further, elevation of extracellular [K+]o from 3 to 6 mM resulted in evoked epileptiform discharge activity (100) and spontaneous epileptiform activity (813) in slices from MAM-aciated rats; such epileptiform activity was rarely observed in controls (evoked: 3%; spontaneous: 0%). These data suggest that prenatal MAM-aciated animals results in functional epileptogenic abnormalities characterized by reduced seizure thresholds. As such, the MAMac model may serve as a useful model for studying early onset seizures resulting from abnormal neuronal migration. This work was supported by NIH grant NS31317.

768.2
FUNCTIONAL CHARACTERIZATION OF THE JERKY PROTEIN IN MICE. G.P. Donovan* and M. Toth, Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

We have identified a mouse gene named jerk which, when disrupted by insertional mutation, causes an epileptic phenotype and EEG. In order to characterize the mechanism by which epileptogenesis occurs, it is necessary to do both localization studies as well as functional analysis of the jerk protein. Computer database searches have revealed significant homology to sequences represented in centromere binding protein-B (CENP-B), Drosophila transposable element POGO-R11, and yeast regulatory proteins Rag3 and Pdx2. The pairwise alignments were most significant with CENP-B and POGO-R11 (P-values < 10^-16). The order of the domains were identical in all sequences, further suggesting the relationship between them. In addition to these homologies, all four of these proteins have DNA binding characteristics. In order to determine if the jerk protein also has this function, we have purified the 41.7 kD jerk protein using a 6-His fusion vector system. We have also epitope tagged the jerk protein at the N-terminus and transfected this construct into mammalian cells for immunohistochemical analysis and further functional studies.

768.3

In the El mouse strain, several genetic loci combine to produce recurrent, tonic-clonic and generalized seizures like common epilepsies in humans. In the mice, seizures occur naturally at ~90 days of age, although for genetic studies we induce ~30 days by gentle rhythmic stimulation to measure seizures quantitatively. Genetic crosses to date reveal several loci: E31 (Chr 14), E12 (Chr 2), EU1 (Chr 9), E13 (Chr 10) and two provisional loci E34 (Chr 9) and E18 (Chr 11). Effects of these loci depend not only on strain background but also on the kind of cross. In a backcross with ABF, EU1, EU2 and EU3 account for most of the difference. But only the minor EU1 had an effect in the corresponding intercross. In an intercross with the DDD strain, a close relative to El, none of these loci had major effects. The big difference between these pairs, El5, maps to Chr 14 accounting for ~35% of the genetic variance. Several minor loci were found, including possibly EU1, and only these were seen in a corresponding backcross to DDD. Finally, in (El x C3H/FP2) intercross progeny, no known El locus was detectable.

Such complexities are enlightening and disturbing. First, they imply there is no single El locus 'essential' for high seizure frequency. Second, although many loci can influence seizures, to explain strain-independent effects these loci must exhibit epistatic interactions. While complex, however, the results are still reproducible within a genetic context. Thus it is possible to not only identify candidates, but to do so in a more rigorous manner with these loci and with large effects by using special mouse strains. For example, we constructed and tested ABP EL-EU1, EU2 and EU3 congenic strains. Compared to ABP, ABP EL-EU2 strain seizures much more, ABP EL-EU3 seizures slightly more, and ABP EL-EU3 no more than ABP. These results are consistent with the relative effects of these loci in prior crosses. We are now testing derivative recombinant strains to find EU2, and in similar studies, EU5.

768.4

Developmental differences in voltage sensitive calcium channel (VS9) subtypes have been discovered in Swiss Webster mouse brain cortical preparations using displacement binding of w-ovaltine(CgTx) GVIA by w-CgTx MVIA (Abbott & Litzinger, 1994). Abbott and Litzinger suggest the presence of a juvenile form of the N-type calcium channel in the developing mouse brain. This juvenile form seems to disappear after dentro-axonal synapse formation during the "critical period" (Esplin, et al 1994) showed that the DBA epileptic mouse whole brain had a different developmental sequence of w-CgTx/GVIA binding. DBA mouse cortex showed the same unusual binding pattern(Jensen, et al submitted, 1995). The potential for aberrant synapse formation in the DBA mouse brain was proposed. The present study compares displacement data from the developing DBA mouse whole brain to Swiss Webster mouse whole brain. At postnatal days 4, 8 and 16, w-CgTx GVIA was displaced by w-CgTx M 91A. Unlike the Swiss Webster data, the DBA showed no juvenile subclasses of the N-type channel at day 8 or day 16. However, preliminary data suggest that the juvenile subtype channel is seen earlier on day 4 and is consistent with the different developmental profile shown in the DBA mouse whole brain w-CgTx GVIA binding.

Neurodevelopment of epileptic DBA mice is clearly different compared to Swiss Webster mice when following markers of synaptogetic calcium channels. Perhaps these developmental differences in the presynaptic calcium channels affect the release of neurotransmitter in the DBA mouse.
DISINHIBITION OF THE DENTATE GYRUS FROM THE EPILEPSY PRONE EL MOUSE REVEALS PAROXYSMAL FIELD DISCHARGES. H. Wang*, W. Frankel and L.M. Menskes, Dept. of Neurology, University of Pennsylvania Medical School and The Graduate Hospital Research Center, Philadelphia, PA 19146 and The Jackson Laboratory, Bar Harbor, ME 04609.

A genetic model of epilepsy, the epilepsy prone EL mouse, develops spontaneous seizures after approximately 90 days of life. Hippocampal brain slices from EL mice were examined during a period of 8-14 weeks of age. Field responses from the granule cell layer of the dentate gyrus during perfusion path stimulation were compared with those from the genetic control, the DMD mouse strain. In control solution, the orthodromic and antidromic responses were similar for the two mouse strains, each response type was characterized by a single population spike, and the presence of a positive field PSP (duration: 10-20 ms) or an absence of a field PSP, respectively. Paired pulse depression recorded at 10, 20 and 70 ms interstimulus interval was significantly less in the EL than the DMD mouse. After exposure to bicuculline (10 μM) for a period of 40 minutes or more, the orthodromic response was distinctly different in the two strains. The response in the DMD mouse (60) exhibited multiple population spikes (2-6x) superimposed on a more prolonged monophasic PSP (duration: 30 ms), whereas, the response in the EL mouse (9) was greatly prolonged (> 400 ms) and was often biphasic with a larger, prolonged negative component following an initial positive potential (69). During the initial 150 ms of the paroxysmal-like response, a rapid population spike discharge (10-50 spikes) occurred. Therefore, the orthodromic response of the dentate gyrus examined in EL mice during a period somewhat before the period of spontaneous seizures was normal in appearance but exhibited reduced feedback inhibition. During disinhibition an underlying paroxysmal excitatory response was revealed which might function later in life during spontaneous seizure generation. Supported by NIH grant # NS23077 to LMM.

THE ROLE OF GABAB LOW-AFFINITY RECEPTORS IN CONTROL OF SEIZURES. I. Velytky*, L. Velytky, M.I. Nunes and S.L. Mons* Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

GABAgentic transmission in the substantia nigra pars reticulata (SNR) controls seizure propagation. In adult rats there are two discrete regions which mediate opposing effects on seizures, bilateral microinfusion of muscimol (both high and low-affinity GABAB receptor agonist) into anterior part of SNR are anticonvulsant, while the infusions into the posterior lateral part are proconvulsant. In 15-day-old rats, the anticonvulsant effect of the proconvulsant muscimol-sensitive network is present. In this study, we determined the role of low-affinity GABAB receptors in the SNR in the control of seizures. In 15 day old rats, bilateral intranigral infusions of ZAPA (a low-affinity GABAB receptor agonist; 2 μg/25 μl) significantly increased the latency to onset of both chronic and tonic-clonic fluroxinduced seizures. In the adult rats, the same doses of ZAPA had no significant effects on either chronic or tonic-clonic flurox-induced seizures in either anterior or posterior part of the SNR compared to the control-infused control.

The data suggest that the low-affinity GABAB receptors in SNR are involved in control of chronic and tonic-clonic seizures in 15-day-old rats, but not in adults.

DECREASED CALCIUM-CALMODULIN KINASE II IMMUNOREACTIVITY IN HIPPOCAMPAL CA1 NEURONS IN AN ELECTRICAL STIMULATION MODEL OF STATUS EPILEPTICUS. A.S. Ross, S.S. Chen, W.J. Ohalu, and R.J. Delauro, Department: Neurology, Department of Physiology, Virginia Commonwealth University, Richmond, Virginia 23298-0594.

The well-characterized hippocampal damage observed following status epilepticus (SE) involves cell loss in the dentate hilar region, and the CA1 and CA3 pyramidal cell layers. The biochemical mechanisms underlying the cell death are hypothesized to be related to alterations in calcium homeostasis. Calcium-calmodulin kinase II (CaMKII) is a calcium dependent kinase involved in many cellular processes such as neurotransmitter release and cytoskeleton formation. Using an ischemia model, this lab has demonstrated a decrease in CaMKII activity which corresponded to a decrease in CaMKII IIβ (60 kDa sub-unit) immunoreactivity in hippocampal regions with subsequent cell death. Since CaMKII IIβ activity also decreases in the continuous high frequency stimulation (CHS) model of SE, we examined the localization of CaMKII IIβ immunoreactivity in CHS treated mice. The CHS paradigm involves 90 min of electrical stimulation (600 μA, 50 Hz in 10 trains every 11s). At 4 h and 3 days post-CHS, all animals were perfused and brain sections were paraffin embedded. Ten micron sections were incubated with anti-CaMKII IIβ monoclonal antibodies (1:500 dilution), followed by an ABC elite kit (Vector Labs) protocol and developed using diamino benzidine and hydrogen peroxide. Positively staining cells were counted per unit length in the CA1 region. Compared to surgical controls the CHS treated animals had decreased (approximately 40%) immunoreactive cells at all time points examined. Loss of immunoreactivity at the early time points without cell loss indicates that the loss of CaMKII II β predicts cells undergoing delayed neuronal cell death.

SELF-SUSTAINING STATUS EPILEPTICUS RESULTING FROM BRIEF PERFORANT PATH STIMULATION IN FREE-MOVING RATS. A. Mazarrat*, Y. Shirakawa*, C.G. Waterhouse, VA Medical Center Sepulveda CA 91343-2099, Dept. of Neurology and Brain Research Institute, UCLA School of Medicine.

We studied the effects of perforant path stimulation (PPS) of various durations on the induction of self-sustaining status epilepticus (SSE), changes in paired- pulse inhibition and brain damage.

Male Wistar rats (13-14 weeks of age) were implanted with a stimulating electrode into the perforant path and a recording electrode into the dentate gyrus and stimulated in the awake state four weeks after surgery (one per minute for 10 minutes of a train of single stimuli at 20 Hz, 20 V, 0.1 ms). After 30 min of PPS, 9 of 10 rats had SSE lasting at least 3 h and the last displayed seizures by ECO without overt behavioral convulsions. 4 rats died within 24 h after PPS. The 5 animals recorded displayed EEG activity and some decrease of paired- pulse inhibition 3 days after PPS. 7 rats had severe bilateral hippocampal damage and lesions in amygdala, pyriform, entorhinal and accessory. 3 had mild or minimal injury. The degree of brain damage correlated with the severity of SSE. 15 min of stimulation induced motor SSE in two of 3 rats and interictal spikes in 1 animal. 1 animal died 24 h after PPS. I rat displayed paired pulse disinhibition 3 days after PPS and in 1 animal paired- pulse inhibition recovered at this time. Neuronal injury was observed bilaterally in hilus, CA1 and CA3. 7 min of PPS did not cause SSE, loss of paired- pulse inhibition and cell loss in any of the rats. These results demonstrate that brief PPS is sufficient to trigger SSE, loss of inhibition and hippocampal damage.

Supported by the VA Research Service and by Research Grant NS15315 from NINDS.

SELECTIVE CHANGES IN GENE EXPRESSION ASSOCIATED WITH EPILEPTOGENESIS IN HIPPOCOMPALENTORHINAL CORTEXICtal SLICES. R.V. Officer, D.A. Coulter, E.L. Jones, and R.J. Delauro, Department of Neurology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23228.

Long term exposure of hippocampal slices preparations to extracellular 0 Mg2+ has been shown to produce recurrent seizures. This study investigated changes in specific mRNA levels that occur in association with these recurrent seizures. Hippocampal/entorhinal cortices (HEC) from male 21-30 days rats were removed, sliced, and placed in oxygenated artificial cerebrospinal fluid (ACSF) in the presence or absence of Mg2+ for 2h. The slices were returned to ACSF containing Mg2+ for 3 h. Epileptogenic activity was elicited throughout representative HEC preparations by a single electrical stimulation. Corresponding slices were frozen, blocked, and cryosectioned for in situ hybridization (Perlin et al., PNAS, 90:1741, 1992). Complementary oligonucleotide probes to the CaMKinase I, II, and III, and the NR2A (150 bp) and NR2B (130 bp) subunits of the NMDA receptor subunits NR1, NR2a, and NR2b were labeled with [α32P]dCTP. Autoradiography was analyzed by a Jandel-Mocha image analysis system. In situ hybridization demonstrated that gene expression of CaMKinase I and II mRNA decreased in the CA1-2, CA3-4, and DG regions. Levels of GABA, GAD and Glutamate in the NMDA subunits for at least 3 h in 0 Mg2+ exposure are consistent with the hypothesis that long term gene changes are induced during epileptogenesis and may contribute to altered neuronal excitability.

Supported by NIH grant RO1-N23350.

Adults with temporal lobe epilepsy are more likely to have experienced seizures in childhood than are adults without epilepsy. Yet it remains unclear whether the primary pathogenetic factor underlying subsequent epilepsy in adulthood is: a) the event or condition which provoked the childhood seizures; b) the seizure itself; or c) an interaction between the two. Perinatal hypoxia-ischemia is a frequent antecedent of pediatric seizures and a not unusual etiology of infantile spasm. In order to evaluate the effects of hypoxia-induced seizures from hypoxia alone, we exposed 10 day old rats to one of two hypoxia protocols. Rats were exposed to 10% O2 for 24 hours followed by one hypoxia-induced seizure or 20 minutes of hypoxia during which the oxygen concentration was manipulated to produce 8 to 12 seizures. Immediate mortality was higher in the hypoxia group (8%) than in the control group (< 1%). Extracellular recordings from hippocampal slices prepared 3 days post-hypoxia showed an increase in field potentials in the CA1 region of slice cultures only for animals from the multiple seizure group. Analysis of cryoultrastained sections through the dorsal hippocampus revealed a significant increase, relative to littermate controls, in both the number and the density of pyknotic cells in the stratum granulosum and hilus of the dentate gyrus in animals from the multiple seizure group but not from the single seizure group. Fluoroxydol testing 60 to 90 days after the hypoxic exposure demonstrated a significant decrease in latency to the first myoclonic jerk and to forelimb clonus in animals from the multiple seizure group, while a lesser reduction was evident in animals from the single seizure group. These results suggest that the seizure episodes contribute significantly to the morphological and functional changes seen in post-hypoxic hippocampus. (Supported by NIH, NINDS grant 15171)

DEXTERN PRESERVATION OF CA3, CA4 AND DENTATE IN HIPPOCAMPAL BRAIN SLICES. H.Q.P. E.Hospital, S. Motawen, S. Trovarelli, A.S. Thompson, Dep. of Neurology and Neurosurgery, SUNY at Stony Brook, NY and VAMC at Northport, NY.

Brain slice models of epilepsy often focus on CA3 and dentate neurons, yet hippocampal slices from adult rats show severe neuronal injury in both areas if incubated submersion in Krebs-Ringer (K-R) at 37°C. In an effort to reduce brain slice swelling during incubations, we have examined the role of dextan, fast-drying shown over 20 years ago to eliminate swelling. We have found that modifying K-R by adding 3% dextan and reducing NaCl by 16 mm significantly improves both histology and water gain. Slices were incubated for 4 h in vitro, submersed 1 mm at 37°C with 1.95±0.05% KCl. Dextan improves histologic score (1-5; lower is better) in all regions, notably CA1 (1.9±0.2 vs. 3.1±2.1) and dentate (1.3±0.7 vs. 2.8±0.6). Wet weight / protein falls from 14.4±3.0% in K- R to 11.9±1.8 with dextan. Water gain over 4 h falls from 33% to 9%. There is no change in tissue ATP, ADP, AMP or energy charge, in the relative size of the extracellular space (0.2%) or the diffusion coefficients for either (3)PEG(4000) (3.0±0.7% c*cm/s) or (2)H2O (1.6±0.9% c*cm/s). Dextan increases glucose utilization from 39.7±1.13 to 78.3±19.5 µmol/min.

Dextan markedly improves histology and reduces water gain of adult rat hippocampal brain slices incubated submersed at 37°C. This is not due to improved adenosine or changes in diffusion but is associated with increased glucose utilization. Dextan should be considered when neuronal instability or obvious water gain is encountered.

Support of VA Merit Review and NIH NS02492 are greatly appreciated.

ABERRANT NEURONAL Firing PATTERNS IN DEEP LAYERS OF SUPERIOR COLLICULUS SUBSERVE AUDITORY SEIZURE EXPRESSION IN GENETICALLY EPILEPTIC-PRIOR RATS. M.E. Randall and C.L. Fangold. Dep. Pharmacology, Southern Illinois Univ. School of Medicine, Springfield, IL 62794.

The deep layers of superior colliculus (DLSC) have previously been implicated in the neuronal network for audiogenic seizures (AGS) in the genetically epilepsy-prone rat (GEPR-9). The present study examined DLSC neuronal responses in chronically implanted microwires. Normal Sprague-Dawley rats and the GEPR-9 were anesthetized with ketamine/xylazine (85/5 mg/kg), and microwire electrodes were implanted into DLSC. Unit activity was recorded at least one week later in freely-moving rats. Responses were evaluated through a variety of acoustic intensities and frequencies ranging from 98 dB SPL (Frey et al., 1990) to over 120 dB SPL (Snyder-Keller and Peterson, 1992), which may elicit differential AGS activity. LE rats at postnatal day 14 (PND 14) were exposed to 10 kHz tone bursts for 8 min. at 120 dB SPL. Beginning at PND 24, subjects (n=16) were tested for AGS using continuous white noise at four intensity levels: 90 dB, 100, 110, and 120 dB, and were recorded while displaying no seizure activity, or while running following clonus. There was a significant main effect of intensity (p<0.001). Post-hoc analysis revealed significant pairwise differences in AGS activity between 100 dB and 110 dB (p<0.001) and between 110 dB and 120 dB (p<0.001). The main effect of gender and the interaction of intensity by gender was not significant.

Another goal of this study was to explore gender differences with regard to seizure onset latency and onset duration. Overall latency for females (M=38.73 s, SD=12.08) was lower than for males (M=31.81 s, SD=14.36, p<0.05). The difference in onset duration for males (M=23.70 s, SD=9.50) and females (M=24.33 s, SD=6.86) was not significant. These data suggest first that AGS activity is intensity-dependent, with 120 dB always eliciting clonus. Secondly, there appear to be some gender differences in AGS characteristics.

(Supported by the Deafness Research Foundation and NSF SBR-002685)
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679.1

SPONTANEOUSLY APPEARING SHARP FIELD POTENTIALS IN HUMAN NEOCORtical SLICES FROM EPILEPTIC PATIENTS: E.L. Speckmann, R. Köhler, A. Frey, H. S. Timm and P. M. Wies, "

679.2

ABNORMAL EXCITABILITY OF DENTATE GRANULE CELLS IN HIPPOCAmPICAL SLICES FROM TEMPORAL LOBE EPILEPTIC PATIENTS: D. Leach, M. O'Conner and A. Paton, "

679.3

MODULATION OF SYNAPTIC RESPONSES IN RAT AND HUMAN DENTATE GRUSS: Anne Willenbring, Karen Kato and Denise O. Spencer, "

679.4

DYNOHRPHIN IMMUNOREACTIVITY IN THE DENTATE GRUSS IN HUMAN TEMPORAL LOBE EPILEPSY: AN ELECTRON MICROSCOPIC STUDY OF REORGANIZED MOSHY FIBER SYNAPSES: N. Zhang and C.R. Houser, "

Society for Neuroscience, Volume 21, 1995
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769.5

CHILDHOOD SEIZURES INDUCE HIPPOCAMPAL NEURON LOSSES AND MOSSY FIBER SYNAPTIC REORGANIZATION. G.W. Mathew * , J.L. Rahn JR., P. Terrazas, J.L. Loie, K.M. Yezierski, P.A. Kuhlman, and W.J. Pronko. UCLA School of Medicine, Los Angeles, California.

This study determined if severe childhood seizures were associated with hippocampal pathology in hippocampal sclerosis from longer seizure histories. Children with catastrophic epilepsy (n=25) and autopsy (n=23) hippocampal specimens were studied for (1) neuron densities, and (2) the density of supragranular neo-Timm's staining. Results showed: (1) Compared to age-matched autopsies, children as young as age 5 years with a history of hippocampal seizures showed markedly decreased granule cell, hilar, and regio superior neuron densities in the pattern similar to adult hippocampal sclerosis. By contrast, children with extra-hippocampal pathologies and seizures showed only decreased granule cell densities. (2) The gray value (GV) densities, when compared to autopsies, showed greater supragranular GV's in children with congenital pathologies, however the GV's in children with hippocampal seizures were greatest. 3) Of the children with extra-hippocampal pathologies there were no statistical correlations between longer seizure durations with changes in neuron densities, or mossy fiber sprouting. These results indicate: 1) Extra-hippocampal childhood seizures are associated with moderate fascia dentata and minimal Ammon's horn neuron losses and signs of aberrant mossy fiber sprouting. 2) By contrast, young children with the syndrome of mesial temporal epilepsy show the neuron losses and mossy fiber sprouting typical of hippocampal sclerosis. These findings support the hypothesis that childhood seizures can damage or alter the postnatally developing granule cells of the human hippocampus, and that early neuron losses and aberrant axon circuits may contribute to chronic hippocampal seizures. However, repeated childhood generalized seizures are not necessarily associated with the development of hippocampal sclerosis. Supported by NS 02080, and K02 NS 1603.

769.7

Localization of the glutamate receptor subunit GluR1 in the hippocampus of patients with Temporal Lobe Epilepsy. R.C. de La Chapelle*, G. van Camp, M. Priez, L. Kovacs, and D.D. Spencer, Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06520.

The GluR1 receptor was localized immunocytochemically in hippocampi surgically removed from patients with intractable temporal lobe epilepsy (TLE). mRNA for the flip and flop splice variants of GluR1 were localized by in situ hybridization with specific radiolabeled oligonucleotides. Excised brains were cut into two broad categories — those that show sclerosis and reorganization (MTLE) and are seizure free, and those that do not show reorganization (mtTLE or PTLE) [Clin. Neurosci., 2, 64-86, 1994]. In the non-reorganized hippocampi, immunoreactivity was weak in the granule cell bodies, but stronger on the apical dendrites throughout the molecular layer (ML). In the reorganized neurons were also stained. Within area CA1 to CA3 immunoreactivity was localized to dendrites and not pyramidal cell bodies. In stratum oriens cell bodies and dendrites were strongly labeled. The flip variant was weakly expressed on granule cell bodies, but strongly expressed on deep hilar neurons, and throughout the pyramid cell layer of area CA3. The flop variant was strongly expressed on granule cell bodies. In the reorganized (MTLE) hippocampi the pattern of immunoreactivity showed two differences. (1) Surviving hilar neurons appeared to have punctate clusters of immunoreactivity on them. Many of these neurons resemble mossy cells. The same pattern of punctate staining occurred on the cell bodies of some CA3 neurons. These patterns of staining may represent receptor expression on these cell bodies or on mossy fiber terminals that synapse on these cell bodies. (2) In area CA1 where there is loss of pyramidal neurons, small immunoreactive neurons appear scattered in the stratum pyramidale. These cells were not seen without pyramidal cell loss. The relevance of these distinct changes in GluR1 expression in the hippocampal seizure focus remains unclear at present. (Supported by T32 N1C 1 N0)

769.9


Tenascin (TNc) is an extracellular matrix glycoprotein transiently expressed by primitive astrocytes in the developing CNS. Its expression appears to correlate with key events during neurogenesis; e.g., neuronal migration and growth. In the adult CNS, TNc is present in the glial scar following CNS injury. In the hippocampus of epilepsy patients with symptomatic temporal lobe epilepsy, TNc immunoreactivity was increased in some patients. This increase was prominent in the dentate gyrus and the hippocampus. In hippocampal specimens from patients with severe neuronal cell loss (Ammon's horn) a striking increase in TNc immunoreactivity was found in all subfields of the hippocampal formation. This increase was prominent in the dentate gyrus molecular layer, the pyramidal cell layer and the stratum radiatum of the Ammon's horn. The staining pattern reflects the distribution of reactive fibroblastic astrocytes in AHS. An upregulation of TNc may be indicative of altered glio-neuronal interactions in the hippocampus of epilepsy patients with Ammon's horn sclerosis and contribute to axonal reorganization and sprouting.

769.6

NEUROPATHOLOGICAL FINDINGS IN STATUS EPILEPTICUS. M.G. Haefeli* C. A. Fortner, R.J. Del carmen. Div. Neuropathology, Medical College of Virginia/VCU Richmond, VA 23298.

We correlated post mortem brain lesions with clinical findings in patients with status epilepticus (SE) (27 adults and 3 children between 2 months and 80 years of age). Clinical features: survival time range from 0 to 59 days post SE. 70% had no prior seizure history; 50% had acute CNS insults; 20% had acute "non CNS" primary causes for SE; 17% had acute hypoxia (anoxia); 9% had preexisting lesions; and 10% were withdrawing from alcohol or antiepileptic drugs. Pathologic changes identified: 23% old or recent infarcts and an additional 37% acute anoxic changes; 19% CNS infections; 13% hemorrhages; 7% contusions; 7% tumors; 7% edema; 5% astrocytosis in various parts of the brain, including 10% in the hippocampus; neuronal loss in 37%, primarily in the cerebellum.

To our knowledge, this is the most comprehensive study to date concerned with morbid CNS changes seen in SE. In particular it provides novel data on SE patients with no prior seizure history in an adult population and those with nonconvulsive seizures. The range and extent of post mortem lesions was much greater than clinically suspected.
769.11 NEOCORTICAL AND HIPPOCAMPAL DEFICITS IN TEMPORAL LOBE EPILEPSY. L. Marsh, P.A. Shear*, E.V. Sullivan, G.M. Morrell, H. Freeman, A. Mann, K.O. Lim, J. Peterfanz, Department of Psychiatry and Behavioral Sciences and Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, and Department of Veterans Affairs Medical Center, Palo Alto, CA 94304

Magnetic resonance imaging (MRI) studies of localization-related epilepsy of temporal lobe origin (TLE) have concentrated on the hippocampus because of the prognostic significance of mesial temporal sclerosis. However, little is known about the extent of volume abnormalities in the neocortex of temporal and extra-temporal brain regions. This MRI study examined whether neocortical volume deficits were present in patients with unilateral TLE, as defined after epilepsy surgery. Regions of interest (ROIs) measured from 3mm spin-echo coronal images, included the hippocampus as well as gray matter of the temporal lobe, superior temporal gyrus (STG), and a frontal-parietal region (FPR). ROIs were measured for patients with normal or abnormal outcome. The results suggested that the hippocampus, as well as gray matter of the temporal lobe, superior temporal gyrus (STG), and a frontal-parietal region (FPR), were affected in TLE. This study may help in the clinical and surgical assessment of TLE.

769.12 AN EVENT-RELATED POTENTIAL (EREP) STUDY OF DISTURBANCE OF SEMANTIC PROCESSING IN TEMPORAL LOBE EPILEPSY. T. Miyazawa
don R. Saegusa
don H. Honma, R. Kobayashi, S. Kohata
don H. Ohkura, and T. Konagaya. Dept. of Psych. and Neurol. and Dept. of Pediatr., Sch. of Med. and Fac. of, Hokkaido Univ., Sapporo, Japan

Several lines of evidence have indicated the disturbance of cognitive function, especially memory and language dysfunction in temporal lobe epilepsy (TLE). We focused the semantic memory which is related to semantic processing in language. We investigated the possible disturbance of semantic processing in TLEs with N400, an event related potential, related to semantic processing. The seven TLE patients were right-handed and aged 17-38 years. All had complex partial seizures and were taking 1 to 3 kinds of anti-epileptic drugs. The controls were matched for handedness, gender and age. We used a category matching paradigm consisting of two conditions; the match-condition in which the target word was the category name for the prime word and the mismatch-condition in which the target was of a different category name from the prime. The subjects were required to press one of two buttons accordingly for matches or mismatches. Reaction times were significantly longer in TLEs than in the controls. The mean amplitudes of N400 in the mismatch-condition in TLEs were smaller than in the controls. This suggests that there is a disturbance of the semantic processing in TLEs, especially in the mismatch-condition.

769.13 ANTICONVULSANT EFFECTS OF RELATLON IN MURINE NO CASE STUDIES. T. Charpentier
don H. Sanchez-Parras
don P. Legros-Huyot
don H. Merle-Bezard
don D. Jurka-Schall
don C. Gattarelli
don J. Thibault
don L. M. Allamand
don N. Thouard
don G. Degueldre
don M. R. B. 77843, Station D. Neuro-Radiol. A. Menarini
don C. C. 77842, Station D. Neuro-Radiol. A. Menarini
don S. S. 77843, Station D. Neuro-Radiol. A. Menarini

Methionin (MEL), a horine produced by the pineal gland, decreases and/or synchronizes neuronal activity in various species suggesting an anticonvulsive capability. The present case studies describe the effects of MEL administration to two children with uncontrolled seizures. The first child is a 32 month old female who has had progressive epileptic seizures since one month of age (15 - 20 seizures per day) who was unresponsive to the known anticonvulsants. The second child was a 2 year old female, who had petit mal seizures with convulsions. Both children were treated with MEL and the anticonvulsants. The second child was unresponsive to the known anticonvulsants. The seizures disappeared. Presently, she is still receiving the MEL and the anticonvulsants. Glutamic acid (GABA) and serotonin (5HT) were increased in both children. MEL was effective in reducing the seizures and improving the social and psychological behavior of the children. This suggests that MEL can be used as an alternative to conventional anticonvulsants.

769.14 ALUMINA GEL INJECTIONS INTO THE AMYGDALE OF MONKEYS CAUSE BEHAVIORAL AND PATHOLOGICAL CHANGES FOUND IN TEMPORAL LOBE EPILEPSY. T. E. Ribak
don L. S. Sershen
don P. Weber
don R. E. Bakay

We investigated the behavioral and pathological changes in the amygdala of monkeys after injections of aluminumsulfate suspensions. The amygdala was injected into the temporal lobes of monkeys to determine whether complex partial seizures could be generated. Time-sustained and electroencephalographic micropipettes were prepared for these injections. The types of pathological changes associated with the generation of complex partial seizures were observed within 2-3 days in monkeys with injections into the amygdala, entorhinal cortex or hippocampus. Hippocampal pathology was limited to the injection site in monkeys with hippocampal injections and was not observed with entorhinal cortex injections. The findings of this study suggest that the amygdala, entorhinal cortex or hippocampus are involved in the generation of complex partial seizures.


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Introduction: The aim of this study was to develop a model of chronic focal epilepsy, in squirrel monkeys. The model was characterized bioelectrically and electrophysiologically, using intracerebral microdialysis and electroencephalography (EEG) respectively.

Methods and materials: Eight squirrel monkeys were used for the experiments. Chronic focal epilepsy was induced by intracerebral injection of 25 ml aluminumsulfate. Intracerebral microdialysis (CM 12 probes, membrane length 2 mm, flow rate of 2.8 µl/min) was performed together with the last EEG recording at the end of the observation period (16-18 months) and the samples were analyzed in order to obtain basal levels for various amino acids.

Results: All animals developed chronic focal epilepsy, persisting during the whole period of observation. The seizures started focally, generalized rapidly and lasted for about 1 minute. Intracerebrally, epileptic spikes were recorded in 7/8 animals 3 months after the injection. The spikes were restricted to the same side as was operated. After 11 months, the interictal episodic activity could also be recorded contralaterally in three animals and ipsilaterally in 4 animals. Basal dialysate levels were elevated in 6 animals. The levels of amino acids were obtained in 6 animals. The levels of glycine and glutamate were significantly higher than normal levels.

Conclusion: Intracerebral injection of aluminumsulfate induces chronic focal epilepsy with spontaneous seizures in the squirrel monkey. This model may be used for exploring various treatments of epilepsy, e.g. noninvasive methods as radiography.
EPILEPSY:

770.1
NMDA RECEPTOR BLOCKERS PARADOXICALLY INCREASE SPIKE-WAVE SYNCHRONIZATION AND NEURONAL EXCITABILITY IN A MUTANT MOUSE MODEL OF SPLENIC EPILEPSY. STARKER, G.K. Nahmod, Nobeles. Developmental Neurogenetics, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The presence of functionalrenoicolic phenotype (SW) activity and the disparity of pharmacological effects on SWdischarges in various models suggest the presence of distinct intervening defects subserving SW epilepsy. It is believed that SW activity is a result of a circuit that includes the neocortex, thalamus and reticular nucleus is converted to anomalous oscillations by enhanced hyperpolarizing GABAergic and depolarizing T-type Ca\(^{2+}\) channel conductance. It has been hypothesized that MK-801 is required within this circuit, since NMDA-R blockers suppress SW discharges in several models. To determine whether NMDA-R blockers can induce seizures in all models, we employed stargazer (szg/szg) mutants (Char. 15) to examine the effects of competitive (CP) and noncompetitive (MK-801) NMDA-R blockade on in vivo SW activity and on in vitro neocortical 0 M\(^{2+}\)-induced epileptiform discharges (0H/A). At doses that suppress SW activity in other animal models, CP (40-60\(\mu\)M) and MK-801 (1.6-2.0\(\mu\)M) paradoxically initiate nearly continuous SW activity in szg/szg mice, while they do not affect EPSPs in wild-type (+/+) mice in field recordings from cortical slices bathed in 0 M\(^{2+}\) ACSF, CP (10\(\mu\)M) and MK-801 (10\(\mu\)M) dramatically increased the frequency ([10\(\mu\)M CP, 311%; 1\(\mu\)M MK-801, 310%; 5\(\mu\)M MK-801, 375%; 10\(\mu\)M MK-801, 411%] and decrease the duration ([10\(\mu\)M CP, 73%; 1\(\mu\)M MK-801, 210%; 5\(\mu\)M MK-801, 92%; 10\(\mu\)M MK-801, 99%] of spontaneous EDS recorded in layer 4 of szg/szg slices. while the abolish or greatly attenuate both these parameters in +/+ slices. These data suggest that NMDA-R activation is not an obligatory step in the final path of burst generation in all models of SW epilepsy, and point to a specific excitability deficit linked to NMDA-R-mediated transmission in szg/szg neocortical cells.

770.3
CORTICAL HYPEREXCITABILITY IN THE SPIKE-WAVE EPILEPTIC MUTANT MOUSE STARGAZER. Enrique D Pauleau, Kai D. Kerguer, and Jeffrey L. Nobeles. Dept. of Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Inherited spike-wave epilepsy arises from aberrant bursting in thalamocortical circuits, but the site of gene-linked hyperexcitability is not known. Recordings were performed in brain slices of adult stargazer mice (szg/szg) and their congenic controls (+/+) Field recordings in thalamocortical slices bathed in ACSF revealed the presence of spontaneous low frequency, synchronous network discharges in all layers of szg but not +/+ neocortices. The mutant discharges were not abolished by section of the thalamocortical projection fibers. Intracellular and whole-cell recordings in cortical layer IV-S neurons showed spontaneous giant depolarizing events generating bursts of action potentials with little after burst hyperpolarization. The after burst hyperpolarization seen in +/+ neurons during bursting induced by 0 M\(^{2+}\) saline was almost absent in szg (6.9±1.1 mV vs 1.5±0.5 mV respectively). In whole brain identification, two classes of regular-spiking neurons were identified in similar ratios in both genotypes: RS (+/+ 16/20; szg 17/22), and SS (+/+ 2/40; szg 5/22). SSg single action (AP) half width, rise time and decay time were significantly increased by +12%, 13% and 12% respectively. The rheobase intensity was significantly decreased by 58% in szg (from 0 30-5 to 25 mV). No differences were observed in single AP overshoot and AHP, afterburst hyperpolarization, resting potential, input resistance or spike duration. The AP spike was significantly increased by 29% in szg (zg 217.7±H/A; +/+ 154.4±H/A). Anomalous rectification (AR) was observed in both genotypes, but a depolarizing "lig" was strongly enhanced in szg (6.6±1.2 mV) relative to +/+ (1.6±0.6 mV). These results demonstrate an increase in network and intrinsic excitability in stargazer cortical neurons that could lower the threshold for thalamocortical oscillations in this model of inherited spike-wave epilepsy. Supported by NIH NS20790 and PHILIPPE Foundation.

770.6

Genetically epilepsy-prone rats (GEPRs) have an innate and widespread deficit in the noradrenergic neurotransmitter system which contributes to their propensity for seizures in response to various auditory stimuli. GEPRs of the mild-seizure strain (GEPR-3s) display enhancement of seizure severity following depletion of norepinephrine (NE) using 5-hydroxydopamine while augmentation of noradrenergic function in the GEPR brain has an antiepileptic effect in the GEPR. Since epileptic propensity in these animals involves NE hypofunction which may result from failure of activation of NE perikarya, the present study sought to discern whether the brain of these animals exhibit activation of the immediate-early gene coincident with seizures. Seizures were induced using a 120 db signal. 2.5 hours following seizures, rats were transcardially perfused and prepared for immunohistochemical processing of brain sections for 5-HT. Quantitative morphometric results demonstrated a profound activation of 5-HT immunoreactivity in neurons of the LC subsequent to seizure. These first suggest that the LC perikarya are highly activated during audiogenic seizures, and perhaps, that deficiencies in other components of the NE system (e.g., terminals) may be responsible for the lack of seizure suppression in the GEPR. Supported by Bayer Pharmaceuticals and SHU.

An expanding body of data has demonstrated that the seizure prone state in genetically epilepsy-prone rats (GEPRs) is partially caused by deficits in central nervous system nonadrenergic transmission. This purpose of this work was to trace these deficits to specific nonadrenergic terminals to one specific brain area, the superior colliculus (SC). Several drugs with different mechanisms of enhancing nonadrenergic transmission were chosen to study. Guide cannula were implanted just above the SC, and bilateral severe autonomous seizures (GEPR-9s) under anesthesia. Five to seven days later, injection cannula were bilaterally inserted into the SC while the animal was awake. The rats were tested for audiogenic seizure intensity at 0.25, 0.5, 1, 2, and 3 h after injection. Bilateral injection of vehicle or prazosin (1 µg/side) or unilateral injection of nisoxetine (0 µg) produced no reduction in the intensity of the audiogenic seizure. Desipramine (2, 4, 8 µg/side), nisoxetine (2, 4, 8 µg/side), and idazoxan (0.25, 1, 4 µg/side) all decreased the seizure intensity in a dose-dependent fashion. Significant decreases in the seizure intensity were also observed after administration of both methoxamine (0.15 µg/side) and phenylephrine (0.15 µg/side). Pretreatment with prazosin (1 µg/side) significantly decreased the anticonvulsant effectiveness of methoxamine (0.15 µg/side) and nisoxetine (0.05 µg/side) and these results suggest that nonadrenergic transmission in the SC may be involved in the seizure regulation in the GEPR model, and that this regulation may be mediated, at least in part, by α1 receptors. (Supported in part by UCMOP-RIR-9442 to QSY).


Cocaine is a local anesthetic that also blocks the uptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT). Because local anesthetics induce seizures in high doses, cocaine has long been believed to induce seizures through its local anesthetic effects. We determined the potency of cocaine to induce seizures in two strains of GEPR-9 rats (DSR; polygenic seizure strain) and Sprague-Dawley (SD) rats, the strain from which the GEPR was derived.

Behaviorally, seizures produced by all 4 drugs were similar. The 4 drugs produced a similar rank order of potency in all 3 strains. Cocaine was the most potent convulsant, followed by bupropion. Imipramine demonstrated intermediate potency. Procaine was by the least potent in producing seizures. Cocaine seizure threshold was lower in GEPR-9s than SD controls. Imipramine and procaine seizure thresholds were also lower in GEPR-9s. Cocaine seizure threshold was elevated in GEPR-9s over SD controls.

Bupropion, imipramine and procaine seizure thresholds were also elevated in GEPR-9s, with the greatest elevation in bupropion seizure threshold (74 percent over SD controls). The resistance of GEPR-9 to the convulsant effects of cocaine as compared to GEPR-9s and SD controls may reflect differences at the level of the DA transporter. The rank order of potency demonstrated by these 4 drugs supports a dopaminergic involvement in cocaine-induced seizures. (Supported by NIH NS 28118.)


Absence seizures represent the synchronized burst firing (spike wave discharges) of populations of cortical and thalamic neurons in an ictal sequence. Previous work in this laboratory has suggested an upregulation of post-synaptic GABA-B receptors in the hippocampus of animals with chronic absence seizures. In order to look directly at intraseizure transmission, whole cell recordings of locally evoked synaptic currents were obtained from CA1 pyramidal cells in coronal slices (450 µm). The peak amplitude of the inhibitory GABAr current measured by GABA(A), (IPSC), and GABA(B) (IPSC) receptors were compared between left hemispheres and the right hemisphere in controls and in animals with chronic absence seizures. The current in the absence seizures was significantly increased compared to controls. In order to block the endogenous activation of excitatory amino acid transmission in the control of generalized seizures, drugs were bilaterally microinjected in the substantia nigra pars reticulata in two genetic models of epilepsy in the rat. The effects of blockade of the nvidia glutamate receptor input from the subthalamic nucleus to the thalamus were then examined.

In Wistar rats with spontaneous absence seizures, bilateral injections of dizocilpine, CGP 40116, or 5.7-dichloroquinoxaline, respectively non competitive and competitive NMDA receptor antagonists and glycine NMDA-associated site antagonists, significantly reduced spike-and-wave discharges in a time and dose-dependent way (600 and 800; 1,000 and 1,000 µg/side). By contrast, no significant suppression were observed after intranarian injections of antagonists acting at other glutamatergic receptors. Furthermore, bilateral injections of muscimol (17.5 and 35 µg/side) in the subthalamic nucleus of C57BL/6J mice suppressed absence seizures in the rat. In a model of convulsive seizures (audiogenic seizure), bilateral intranarian injections of CGP 40116 (20 and 40 µmol/side), resulted in a suppression of tonic seizures. No suppressions were observed after bilateral microinjections in the subthalamic nucleus of muscimol at doses up to 700 µmol/side. These results show that blockade of NMDA receptors within the substantia nigra pars reticulata was effective at both convulsive and non-convulsive seizures in the rat. Furthermore, the suppression of absence seizures after inhibition of subthalamic neurons suggests the involvement of the indirect striato-nigral pathway in the triggering of the nigral control of epileptics.
Epilepsy: Genetinc Models


The DBA/2 mouse is known for its extreme susceptibility to audiogenic seizures. These seizures are triggered by intense, high frequency sound and follow a stereotyped course beginning with a wild running phase that may rapidly progress to clinical seizures, tonic extension and death. Seizures appear to be the result of an imbalance between excitatory and inhibitory transmission in DBA/2 mice. Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system. Thus, a block of glutamate release or receptor binding may prevent or attenuate seizure activity in these mice. We tested the efficacy of the noncompetitive NMDA antagonist MK-801 and CNS 1102 in this assay. Both are highly effective at suppressing audiogenic seizure activity; MK-801 at a dose of 0.25 mg/kg, ip and CNS 1102 at 2.0 mg/kg, ip. The relative in vivo potencies of these compounds closely match their relative in vitro potencies at the NMDA receptor, suggesting that blockade at this receptor is responsible for seizure suppression. For pretreatment times up to 4 hours, CNS 1102 significantly reduced seizure severity in this assay compared to controls, whereas MK-801 was less effective. We then examined 4 compounds that are believed to block the synaptic release of glutamate: lamotrigine, ritanserin, BW697C89, and CNS 1237. All were effective at reducing seizure incidence and severity, although at higher doses than were required for NMDA antagonists. The DBA/2 mouse audiogenic seizure model is a rapid and highly reproducible method of assessing a compound’s ability to block glutamatergic excitation. The ability of both NMDA antagonists and inhibitors of glutamate release to prevent audiogenic seizures suggests these seizures may be glutamate-mediated. Drugs selected with this model may have therapeutic value not only for the treatment of epilepsy, but also for other disorders associated with glutamate excitotoxicity including stroke and traumatic brain injury.

T70.15 MIDBRAIN 3D DIPOLE MAPPING OF ACOUSTIC EVOKED POTENTIALS IN AUDIGENIC SEIZURE RESISTANT ANESTHETIZED RATS. Morgan, M.J.S.P., Baber, A., Del Vecchio, F. and Garcia-Carreno, N. Neurophysiology and Experimental Neurophysiology Laboratory, Department of Physiology, Ribeirao Preto School of Medicine, University of Sao Paulo, Ribeirao Preto, 14045-900, S. P. Brazil.

The inferior colliculus (IC) is the most critical structure of the auditory midbrain involved in audiogenic seizures (AS), a model of experimental epilepsy. AS are induced in susceptible (S) rats, in contrast to resistant (R) rats, by high intensity acoustic stimulation. Thus, in order to deduce functional differences in S and R ICs, we began mapping wave form patterns of midbrain acoustic evoked potentials (AEPs) in R Wistar rats. Data were collected from anesthetized rats (thioneronal 40 mg, 240-280 g, p.w. by g, of carbon fiber electrodes)) from 5 ICs, developed in our laboratories, located in the following coordinates, at 0.5mm intervals: A/P(anterior) from +0.5 to 2.0mm, (Medline) from 0.5 to 3.0mm, (Ventral mesh) from 1.5 to 7.0mm, therefore, the mapping procedure resulted in 360 recorded signals [5 x 72 x 10] applied to the coordinate plane. The collected AEPS were pronounced (p<0.001) by means of an A/D converter interfaced with a computer(TEAC) and the data expressed as a percentage of maximum of each lateral sweep. Small electrical impulses labeled recording points. The results of AEP mapping are presented as conventional 20 ms recordings and used to calculate instantaneous electrical dipole in each 0.5mm control volume. The calculated dipole enabled us to reconstruct a cube of the mesencephalon in which the IC is inserted. Results of 30 minutes reconstruction from 0 to 20 ms on a 1ms time interval suggest the signal coming in at 3 ms through the lateral lemniscus, 5 ms in the IC; in 6 ms the signals split to the superior collicus, external and dorsal IC nuclei and also projects to the contralateral IC. At around 8ms, central IC seem to receive feedback information from external nuclei. After 9 ms, no significant dipoles are recorded in the cube. Ongoing studies are highlighting differences and similarities between the dynamic (temporal dependent) 3D dipole distribution of S and R rats.

Financial Support: FAPESP - Brazil (grant # 93/02322-2)

T70.17 PROPERTIES OF DEVELOPING RAT HIPPOCAMPUS CA1 NEURONS IN THE GENETICALLY EPILILEPTIC PRONE RATS (GEPKRS). L. Vorma-Ahaja, T. L. Probst and M. S. Evans, Department of Surgery, Division of Neurosurgery and Department of Neurology, Southern Illinois University, School of Medicine, Springfield, Illinois 62704

The developing GEPKRs like normal young rats show a greater propensity for tonic seizure induction during the first two weeks of postnatal development. In the third and fourth weeks, the GEPKRs lack the developmental decrease in tonic seizure susceptibility that occurs in normal rats. The adult GEPK hippocampus shows an increased excitability in the CA1 region with paired pulse stimulation, a decreased GABAA-mediated inhibition, reduced spike frequency adaptation with a reduced slow afterhyperpolarization (AHP) in some neurons. We now studied the properties of developing hippocampal CA1 neurons in GEPKRs (n=32) and SD rats (n=9). The membrane input resistance was higher in the developing CA1 neurons in GEPKRs. The fast AHP was absent in GEPK and the repolarization was slower. The slow AHP was not significantly different in developing GEPK CA1 neurons. All the cells studied in the CA1 region in the SD rats during the first three weeks showed a spike frequency adaptation. In 40% of the GEPK CA1 cells recorded in the 3rd or 4th week of development, the spike frequency adaptation was absent. On single synaptic stimulation, some of the CA1 cells showed burst of action potentials in the 3rd week. Decreased inhibition with repetitive stimulation was seen in developing GEPK rats. Single synaptic stimulation in the presence of 10 nM GABA concentrations lasting about 30 ms in SD rats at three weeks. In GEPKRs, however, a prolonged discharge lasting for up to 250 ms was seen in CA1 neurons. These results show an increased excitability in the developing GEPK CA1 neurons at the time when behavioral seizures are beginning to appear.

T70.14 LAMOTRIGINE DRAMATICALLY DECREASES CONTENT OF DOPAMINE METABOLITES IN CAUDATE NUCLEUS OF NORMAL AND SEIZURE-PRONE BALB/C MICE. J.F. Yiend*, and N.A.M. Alackin. Department of Anatomy, University of Manitoba, Winnipeg, Canada R3E 0W3.

Lamotrigine (LTG) is a relatively new anticonvulsant agent. Little is known concerning its neurochemical mechanism of action. In our laboratory we tested LTG for its anticonvulsant effects in an audiogenic seizure-prone strain of Balb/c mice. The initial part of this project was designed to study the neurochemical effects of LTG at clinically relevant doses. A report of its use in Parkinson's disease (Zipp et al., 1993) led us to examine the effects of LTG administration on the content of dopamine (DA) and its metabolites in the striatum. LTG administered 40 minutes prior to behavioral testing, inhibited tonic-clonic audiogenic seizures in a dose-dependent fashion. Complete inhibition of seizures was observed at doses between 10 and 20 mg/kg. At these doses, LTG had no significant effect on DA content in extracts of caudate nucleus microspins. However, LTG administration significantly reduced the content of the DA metabolites, DOPAC and HVA, in caudate nucleus of both seizure-prone and seizure-resistant mice. DOPAC concentrations were reduced to 43% (p<0.001) of controls in seizure-resistant mice, while a reduction to 34% (p<0.001) of controls was observed in the seizure-prone strain. HVA concentrations were reduced to 81% (p<0.05) in seizure-resistant mice, while a reduction to 56% (p<0.001) was observed in seizure-prone mice. While these data do not provide evidence for a role of DA in the anticonvulsant action of LTG, the dramatic effects of this drug on the content of DA metabolites suggest that LTG is an inhibitor of DA turnover. An alternative explanation is that LTG reduces intracellular levels of metabolites by inhibiting reuptake of DA and its subsequent metabolism to DOPAC and HVA. A question that arises from this work is whether the inhibitory action on DA metabolite content is secondary to an action on glutamergic or GABAergic neurons.

T70.16 EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION IN DIFFERENTIAL EPILEPTIC SEIZURE MODELS. Cel Bot E.A, Oliveira P.R., Oliveira J.A.C, Metra J.P.K, Jobe P.O.C, Garcia-Marques, M. Physiology Department, Ribeirao Preto School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil. Department of Basic Sciences, College of Medicine at Florida, University of Illinois, Illinois, USA.

Discrepan results have been found concerning the role of nitric oxide (NO) in epileptic seizures. Inhibition of endogenous NO production is an adequate tool for establishing a role for NO in brain excitability. We evaluated the effect of a NO synthesis inhibitor (L-NOARG), 25 mg/kg, twice a day, 4 days and 125 mg/kg, ip, 30 min before seizure (or drug injection), in pilocarpine, penylentetraazol (PTZ) and audiogenic seizures (AS) on nontreated rats (R) and genetic epilepsy-prone rats (GEPKRs-3s) and Sprague-Dawley controls (CDs). Eighty percent of the rats (X = 0.05) had seizures elicited by a sub-convulsive dose of pilocarpine (100 mg/kg, ip, 1 mg/kg methyl xacloprine, n=8 per group), only after NOS inhibition. NOS inhibition had no effect on sub-convulsive doses of PTZ (15 and 30 mg/kg, n=8), but potentiated the severity of limbic seizures induced by PTZ 60 mg/kg, n=16. Moreover, the L-NOARG treatment protected against tonic seizures and lethally induced by PTZ (60 mg/kg, n=8). L-NOARG treatment had no effect on eliciting AS (8-10 per group) in Wistar rats other than 30 min or 24 h after L-NOARG inhibition. Moreover, GEPKRs-3s and CDs did not modify their severity indexes or seizure latencies after similar treatments. In conclusion, these results suggest that the effect of NO inhibition on seizure activity depends on the seizure inducing agent and in the case of chemical induction (i.e. PTZ) it depends also on the dose.

Financial Support: FAPESP-Brazil (grant # 92/4466-3 and 93/30323-2)
T71.1
GABA-LIKE IMMUNOREACTIVITY (GABA-LI) IN MOSSY FIBERS OF HIPPOCAMPAL BRAIN SLICES. R.S. Wilson, M. Price, B. Johnson, R. SlivSter, & M.A. Dichter. Depts. of Neurology and Pharmacology, University of Pennsylvania, Philadelphia, PA, 19104 and Helen Hayes Hospital, West Harrison, NY 10590

Dentate granule cells provide the main excitatory input to the CA3 pyramidal cells of the hippocampus and are thought to utilize glutamate as a transmitter. However, recent work (see SlivSter et al., this meeting) demonstrates that dentate granule cells normally contain both GAD-67 and GABA-LI. When perforant path stimulation is used to induce seizure activity, granule cell GAD and GABA staining increases selectively. We were interested in reproducing this phenomenon in the acute hippocampal slice. Rat hippocampal slices (400-500 µM) were placed in oxygenated Ringer solution at 33-34°C for varying lengths of time. Some slices were incubated in Ringer solution lacking Mg²⁺ to induce seizure-like activity, while others were placed in either standard Ringer or Ringer containing CNQX and AP5 to block excitatory neurotransmission. Following incubation, sections were fixed with 4% paraformaldehyde and cryoprotected by sucrose. Brain sections (10 µm) were stained with standard protocols for immunoperoxidase with a monoclonal GABA antibody (Sigma). Slices that were fixed immediately after dissection showed GABA-LI in the mossy fiber terminals, while brain slices incubated under all other conditions demonstrated more intense GABA-LI in the terminals and throughout the mossy fiber pathway. These findings confirm that GABA-LI is present in the mossy fiber terminals under baseline conditions, and that merely incubating slices in standard Ringer solution, a common protocol in laboratories using hippocampal slices for electrophysiological and biochemical studies, is sufficient to greatly enhance GABA-LI in the mossy fiber pathway.

T71.3
EARLY GANODACETODIA MODULATES LONG-TERM POTENTIATION AT SCHaffer COLlaterAL-CA1 SYNAPSES IN HIPPOCAMPUS. L. Yalcisk, J. Valaskova, A.M. Egan, D.J. Mohler, P.K. Somitto. Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Gonadal steroids play an important role in the development of the nervous system. In this study, we examined the gonadectomy-induced changes in Shaffer collateral-evoked neurotransmission in CA1 hippocampal slices in slices from 31-day-old male rats castrated on day of birth. Sham operated littersates served as controls. In another study, we examined slices from adult females ovariectomized 7 days prior to the experiment, and given estrogen supplement two days prior the experiment and a single progesterone injection at day of the experiment. We observed no differences in the maximal population spike amplitude between naive slices from normal and castrated males. In slices from ovariectomized females given hormone supplement we found significantly larger maximal population spike amplitude than in controls (86.2±1.3 mV versus 62.7±0.7 mV). In both cases, changes in gonadectomy rates exhibited significantly larger short-term potentiation-1 to 20 min following tetanization, compared to controls. In contrast, gonadectomy did not cause any difference in the amplitude of LTP measured 30 min after high-frequency stimulation (2x100Hz,5sec) in males or females.

The data suggests that gonadectomy causes a selective enhancement of short-term potentiation of synaptic transmission in hippocampal field CA1.

T71.4
CONTINUOUS HIPPOCAMPAL STIMULATION-INDUCED STATUS EPILEPTICUS PRODUCES GABAERGIC INHIBITORY MODULATION IN MICROGLIAL CELLS IN PTZ-INDUCED KINDLING. S. Onbntn, L. Kehayama, T. Ohashi, T. Watanabe, T. Ito, H. Kato, F. Arakawa. Medical College of Virginia, H.S. 803, Richmond, VA 23298

Status Epilepticus (SE) is a seizure disorder associated with high mortality and morbidity and neuronal cell loss despite aggressive treatment. Hyperexcitability and neuronal cell death have been well correlated with kindling-induced seizures in intracerebral cortex. This has provoked interest in calcium homeostatic mechanisms such as the high affinity, low capacity microsomal Ca²⁺ ATPase. Alterations of this enzyme could contribute to the prolonged increases in intracellular calcium that ultimately accounts for the high morbidity and mortality associated with prolonged seizures. The Continuous Hippocampal Stimulation (CHS) model of SE was employed to examine the effects of seizure activity on microsomal calcium uptake. Test animals revealed “continuous” hippocampal stimulation consisting of 104 trains of 50Hz, 1ms biphasic square wave pulses (∆040µA) every 11s for 90 minutes. Implanted/sham animals were used as controls. Rat brain microsomes were isolated from control and SE animals by differential centrifugation for determination of microsomal calcium uptake. Microsomes prepared from control and SE animals displayed ATP-dependent Ca²⁺ uptake. However, CHS-induced SE resulted in significant inhibition of Ca²⁺ uptake, 47%, compared to control. The data indicate that SE causes alterations in microsomal calcium uptake and thus may play a role in causing elevated intracellular Ca²⁺. The data support the hypothesis that alterations in microsomal calcium uptake may ultimately account for some of the morbidity and mortality associated with prolonged seizures.

T71.5
FELBIMATE PREVENTS THE DEVELOPMENT OF KINDLING PRODUCED BY CHRONIC TREATMENT WITH PENTYLENETETRAZOL IN THE RAT. M. Orlindt, G. Giorgi, F. Maroni, M. Spiga, V. Valedonni and M.C. Cardelis. Departments of Toxicology and Neurology, University of Cagliari, Italy.

Chronic daily subcutaneous dose of pentyleneetetrazol (PTZ), a blocker of the Cl channel of GABA receptors, determines the progressive development of seizures (i.e., chemical kindling). It has been proposed that an enhancement in NMDA receptor-mediated neurotransmission together with a decrease in GABAergic function play a role in PTZ-induced kindling. (J. Pharm. Exp. Ther. 262, 782, 1992). Since the anticonvulsant effect of Felbimate (FELB) is probably mediated via interaction with the glycine site of NMDA receptors, it was of interest to determine the ability of FELB to antagonize PTZ kindling. Rats were treated with a subcutaneous dose of PTZ (30 mg/kg, i.p., every second day) for up to 8 weeks. Two groups of rats received FELB (300 or 400 mg/kg, i.p., 10 min before each dose of PTZ) and control rats were treated chronically with saline (2 ml/kg, i.p.). Pretreatment with FELB prevented the development of PTZ kindling partially at the dose of 300 mg/kg and completely at 400 mg/kg seizure score by the end of the chronic treatment, 5s scale: control, 0; PTZ alone, 3.25; PTZ + FELB (300), 1.13; PTZ + FELB (400), 0.15 to 0 days. PTZ-induced increased sensitivities to the convulsant effect of GABA function inhibitors observed in PTZ-kindled rats. Thus, a challenge dose of isoniazid (120 mg/kg, s.c.) or PTZ (30 mg/kg, s.c.) at the end of the chronic treatment induced generalized convulsions in >80% of PTZ-kindled rats and in <10% of controls or rats treated with PTZ + FELB (400). The results suggest that PTZ-induced changes in neurotransmission may be involved in the development of PTZ kindling.

T71.6
LIMBIC SEIZURES CAUSE CHRONIC SUSCEPTIBILITY TO STRESS IN RATS. J. Wang², S. Bres-Elkaïched², G. Tissir², M. Mustier². Psychology Research Unit, Department of Psychology, Tel Aviv Univ., Tel Aviv, Israel.

Some patients with temporal lobe epilepsy (TLE) experience psychiatric symptoms during the interictal period of the disorder. Study of such interaction may further our understanding of psychiatric behavior. Electrical kindling in animals, a process by which chronic stimulation produces marked hyperactivity, is one of the full clinical tonic limbic seizures, is used as a model for TLE. The long term effects of kindling include a decrease in dopamine (DA) reactivity in the prefrontal cortex and increased DA reactivity in the striatum. Such cortical-striatal pattern of DA imbalance may underlie some psychiatric behaviors. Such pattern also predicts increased behavioral reactivity to stressful stimuli, a complaint among psychotics. Therefore, in the present study, rats were electrically or sham stimulated once daily until 5 or 10 full tonic clonic seizures have occurred, and their susceptibility to stress was tested 4 weeks later. Kindled animals compared to sham-kindled and naive controls, showed increased locomotion in a novel environment and elevated analysis on a hot plate test. (15 °C) did not abolish the effect of kindling on the analgesic response. To further study the relationship between kindling and stress, binding to striatal DA-1 (5CHR-25390) and DA-2 (5Y-99153-2) receptors and to DA-1 and DA-2 receptors (5CHR-12935) was assessed using autoradiography following acute restraint stress. Acute stress caused increased DA-2 receptor binding, but not in DA-1 receptor. However, neither kindling nor the interaction of kindling and stress had significant effect on binding to DA receptors or DA uptake sites. In conclusion, the present result suggests that kindling-induced changes in neurotransmitter system underlying this effect is not related to changes in DA receptors or DA uptake sites.
771.7


Excitatory amino acid receptor antagonists are anticonvulsant in a variety of seizure models. We studied the effect of two non-competitive NMDA receptor antagonists on the latency to electroencephalographic discharges in awake rats (male, Sprague-Dawley, 380-480 g) exposed to 5 atm O2. MK-801 (0.05 to 4 mg/kg) or ketamine (K) (20 or 100 mg/kg) (mib) injected ip 30 min before exposure markedly delayed the latency to seizures when compared to controls (C) (n=8) (Fig. 1). Because MK-801 and ketamine may increase cerebral blood flow (CBF), and increases in CBF may enhance O2 availability, we investigated the effect of MK-801 on CBF. We chronically implanted a laser-Doppler flow probe over the dura of a separate group of rats (n=4) of 5 atm O2. MK-801 progressively increased CBF up to 300% between 0.05 and 1 mg/kg. The effect of MK-801 on CBF correlated with the increased CBF. The proconvulsant effect was slightly attenuated at higher doses (> 0.2 mg/kg) known to be effective in other seizure models. We propose two mechanisms by which MK-801 effects O2 seizures: (1) A dominant proconvulsant effect mediated by increases in CBF and (2) A less-powerful anticonvulsant effect operating at higher doses via a receptor-mediated mechanism. (Supported by NIMHD Work Unit 61135N MR000613-1501)

771.9

MODIFICATION OF KAINATE-INDUCED BEHAVIORAL AND ELECTROGRAPHIC SEIZURES FOLLOWING INHIBITION OF NITRIC OXIDE SYNTHASE IN MICE D.D. Kithy*, R.A. Finklea, and S. Subramaniam. Neurological Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892-1408 and Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

We assessed the effects of N6-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, on kainate-induced seizures in adult male NIH Swiss mice. L-NAME dose-dependently decreased shortened latency to convulsions following systemic kainate (44 mg/kg, s.c.) and increased the incidence of convulsive wild running (to the exclusion of the less severe clonic convulsions typically seen in controls). L-NAME did not shorten latency to convulsions induced by intraperitoneal injection of L-glutamate, kynurenic acid, or 6-OHDA in the hippocampus, amygdala, frontal cortex, or midbrain reticular formation. Also, 4 of 5 L-NAME-treated (15 mg/kg, ip) mice failed to express AD during initial fits of wild running, suggesting an uncoupling of electrographic and behavioral components of kainate-induced seizures. Finally, L-NAME shortened latency to wild running induced by intraventricular kainate (1 mmol) but altered neither behavioral change (AD) in the hippocampus, amygdala, frontal cortex, or midbrain reticular formation. In mice, the effect of L-NAME on kainate-induced seizures was converted by intraperitoneal kainate (1 mmol). Because L-NAME potentiated seizures following either systemic or intraventricular administration of kainate but not intraperitoneal kainate administration, the proconvulsant effects of L-NAME may depend on extracellular or hippocampal actions of kainate. Moreover, because L-NAME facilitates kainate-induced wild running, which bears striking similarities to motor components of audiogenic seizures in susceptible strains of rodents, it is possible that L-NAME influences tectopontine mechanisms that mediate audiogenic seizures.

771.11

INCREASED PURINE LEVELS IN THE HIPPOCAMPUS MEASURED BY MICROWAVE ISOLYSIS FOLLOWING SEIZURES. R.P. Varnum* and R.E. Henning. Department of Anesthesiology and Pain Medicine, Wayne State University, Detroit, MI and Karolinska Institutet, Stockholm, Sweden.

There is pharmacological evidence that adenine is an endogenous neuroprotectant agent. We therefore measured brain levels of purines (e.g., adenine, inosine, guanine, xanthine, and hypoxanthine) following focal cerebral seizures. Seizures were produced in anesthetized male rats by injection of either bicuculline (0.5 mg/kg, i.v.) or kainic acid (12 mg/kg, i.v.), and were monitored electroencephalographically. An electrode implanted unilaterally, into the hippocampus. A 10 mm microdialysis probe was implanted in the left hippocampus. Purine levels were quantified by HPLC. Levels of purines increased substantially during seizures, with the largest increases seen for hypoxanthine and inosine. Smaller increases were seen for adenine. These effects were observed with both kainate and bicuculline, but only following seizures that are not followed by seizures. Seizures were produced in anesthetized male rats by injection of either bicuculline (0.5 mg/kg, i.v.) or kainic acid (12 mg/kg, i.v.), and were monitored electroencephalographically. An electrode implanted unilaterally, into the hippocampus. A 10 mm microdialysis probe was implanted in the left hippocampus. Purine levels were quantified by HPLC. Levels of purines increased substantially during seizures, with the largest increases seen for hypoxanthine and inosine. Smaller increases were seen for adenine. These effects were observed with both kainate and bicuculline, but only following seizures that are not followed by seizures. Seizures were produc

771.12

PATTERN OF DISTANT NEURONAL DAMAGE INDUCED BY AMPA-RECEPTOR MEDIATED STATUS EPILEPTICUS EVOKED FROM AREA TEMPESTAS. A. Easton*D. Belcher, D. Mesko, K. Galvan, N. Shahbaba. Georgetown University Medical Center and University of DC, Washington, D.C.

Cyclohexadine (1.8 mg/ml), an agent preventing desensitization of AMPA receptors, when focally applied concurrently with bicuculline methiodide (1.8 mg/ml) into the area of a permanent focal excitotoxic lesion (7.8 Hz) status epilepticus (SE) which can be terminated by application of an AMPA receptor antagonist, NMDA (500 mg/ml) in AT. To evaluate the pattern of neuronal injury in this model, rats were sacrificed 2 days after SE and the brains processed using silver impregnation. With SE lasting 2-3 h, pronounced neuronal degeneration occurred bilaterally in a variety of forebrain structures including the olfactory nuclei, amygdaloid nuclei, insular, and cortical areas. The pattern of degenerative changes was similar to that observed following systemic kainic acid. Rats with brief periods of SE (1 h), neuronal damage was largely restricted to the hemisphere from which the seizures were initiated. Under these conditions, there was no apparent evidence of degeneration in the hippocampus. Otherwise, the brain regions showing the highest degree of neuronal degeneration were those affected bilaterally in rats with more prolonged SE. It appeared that involvement of the hippocampus was closely associated with, and possibly required for, degeneration of regions in the contralateral hemisphere. These results show that SE evoked focally from AT leads to a pattern of brain damage resembling that observed following systemic convulsant

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771.13 BOTH EXCITOTOXIC AND APOPTOTIC MECHANISMS MAY UNDERLIE BRAIN DAMAGE INDUCED BY INJECTION OF AMINOACETIC ACID INTO THE RAT ENTORHINAL CORTEX. W. Dus* and T. Eid. *Maryland Psychiatric Research Center, Baltimore, MD 21228 and 1Department of Anatomy and Cell Biology, University of Bergen, Bergen, Norway.

Injection of aminoacetic acid (AOAA), an indirect excitotoxic, into the rat entorhinal cortex (EC) causes a seizure-related neuronal loss in EC layer III (E. J. Cell Biol. 119:921, 1992). In order to determine whether this distinct neurodegeneration involves apoptosis, we examined AOAA-injected rats using Nissl staining and an in situ DNA nick-end labeling technique (TUNEL. J. Cell Biol. 119:921) at 24 or 48 hours, or 5 days after the AOAA injection. AOAA treatment resulted in intense nuclear TUNEL labeling in neurons within several limbic regions ipsilateral to the injection. At 24 hours, label-positive neuronal nuclei were mainly detected in the most medial region of layer III in the ventro-MEC and in the lateral amygdaloid nucleus. At 48 hours, many label-positive neurons were observed not only in layer III of the entire MEC and in the lateral amygdaloid nucleus, but also in the subiculum. This distribution pattern of TUNEL-labeled nuclei remained similar after 5 days. Moreover, morphological characteristics of apoptosis, such as condensation of chromatin and nuclear fragmentation, were noticed in the regions described above. Our results indicate that, in addition to the excitotoxic mechanism previously suggested, a delayed apoptotic cell death is involved in AOAA-induced neurotoxicity in limbic regions.

The functional consequences of the dual mechanisms underlying these AOAA-induced lesions are currently under investigation.

Supported by a grant from the Epilepsy Foundation of America.

771.14 INHIBITION OF MICROGLIA ACTIVATION REDUCES CORTICAL NEURONAL CELL LOSS AFTER STATUS EPILEPTICUS. L.S. Chen and G.C. Smart III. Div. of Neurology, Children's Hospital Los Angeles, University of Southern California. Los Angeles, CA 90027

Status epilepticus (SE) is associated with acute neuronal injury, which can activate microglia. The activated microglia are capable of killing neurons by releasing neurotoxic factors. Therefore, seizure-induced neuronal cell loss (SICL) may have two components: seizure-induced primary NCL and microglia-induced secondary NCL. To test this hypothesis, we studied the effect of inhibiting microglia activation on NCL after SE. Rats were given intravenous injection of 0.1 umole kainic acid to induce SE or vehicle for control (n = 6). Neuronal cell density (NCD) was calculated in different cortical layers. NCL was calculated as: the difference of mean NCD between experimental and control groups/mean NCD of controls. Microglia were identified by C3 receptor (OX-42) immunohistochemically. SE produced progressive cortical NCL. The NCL was 49% (n=6) at day 1, 67% (n=6) at day 3 and 96% (n=6) at day 7. Despite the cessation of seizures at day 1 or day 2, the progressive NCL was correlated with a progressive increase of microglia in the cortex. Administration of colony-stimulating factor (0.2 mg/kg/day, i.p.) and chloroquine (2.5 mg/kg/day, i.p.) decreased OX-42 immunoreactivity and reduced NCL (p < 0.01). The NCL was 36% at 7 days after SE in rats (n=5) treated from day 1-4, and was 51% in rats (n=6) treated from day 4-7. These data suggest that microglia activated after SE significantly contribute to NCL. Early intervention to inhibit microglia activation can reduce NCL after SE.

771.15 REGENERATION AND HYPEREXCITABILITY IN HIPPOCAMPAL CULTURES AFTER TRANSSECTION OF THE SCHACER COLATERAL. R.A. McKinney, B.H. Gáthiver and S.M. Thompson. Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

Seizures are a common consequence of head trauma, and it has been suggested that this neuronal reorganization may contribute to lasting changes in excitability. Mature organotypic hippocampal cultures (>14 days in vitro) were used to investigate the capacity of recurrent axon collaterals of CA3 pyramidal cells to sprout in response to transection of the Schaffer collateral pathway with a razor blade. Cells were allowed to survive for varying times before either injecting biocytin, to observe axonal arborization, or performing GAP-43 immunohistochemistry to visualize regenerating axons. Quantitative changes in dendritic spines were investigated by injecting CA1 cells with Lucifer Yellow and imaging by confocal microscopy. Axonal sprouting within area CA3 was evident at 3 days post-injury. By 14 days many axons possessed growth cones and were found to have grown across the cut to the CA1 region. At 3 days post-trasection, dendritic spines on CA1 cells were elongated and their number began to diminish by 7 days post-injury. Interestingly, spine number returned to control values by 21 days. Functional synaptic excitation of CA1 cells by stimulation in area CA3 was recorded at >14 days post-injury. Sprouting axons exhibited more swellings, i.e. putative presynaptic boutons, compared to control axons. Evidence of an increase in the strength of excitation relative to inhibition within area CA3 was apparent, thereby suggesting a possible role for axonal sprouting in post-traumatic epilepsy.


When spike-wave (SW) paroxysmal activity spontaneously develops from sleep patterns in cortical/thalamic circuits in unrestrained cats, a surprisingly high number of thalamocortical (TC) neurons are hyperpolarized and remain silent throughout the seizure (Contreras and Contreras, 1995). To shed further light on the behavior of such TC cells, we performed intracellular recordings of thalamic and cortical neurons and simultaneously recorded the surface- and depth-EEG activity from the related cortical areas. During seizures, the membrane potential of TC cells oscillated between rhythmic EPSs, in close temporal relation to the depth-positive EEG wave. This regularization decreased with depolarization, and was enhanced by hyperpolarizing DC, thus suggesting a non-GABA mediated mechanism. The regularization was not enough to de-inactivate the Ca++ current underlying spike-bursts and most TC cells remained silent. With slight hyperpolarizing DC injection, cells fired typical spike-bursts at each depth-positive EEG wave (see figure). With depolarizing d.c. we fired short spike-trains that could be easily confused with spike-bursts in extracellular recordings. We propose that the absence of burst-firing in a large proportion of TC cells is due to the lack of a GABAergic component characteristic for other forms of SW seizures. Supported by MRC of Canada (grant MT-3681) and the Savoy Foundation.


We describe the application of a custom designed parallel processor in real time digital signal analysis in experimental epilepsy. Our main goal is to analyze the temporal characteristics and propagation of afterdischarges induced in the temporal lobe amygdala to the cerebral cortex. This processor is able to generate a "cartoon" of the propagation dynamics of the afterdischarge with a very high temporal resolution, and study the evolution of each spike in the cortex. As the kindling develops, the afterdischarge becomes wider and complex in terms of the dynamics of propagation.

The method generates a series of color maps which show the spatial evolution of a selected afterdischarge. To satisfy the computational requirements, we design a parallel processor based on 4 DSP (digital signal processor) chips with a total 80 million instruction per second. Thus can be generated one map in millisecond, allowing the real time tracking of ical and interictal phenomena. Each processor also has 4 analog input channels. This scheme yields an efficient process distribution among processors resulting in a very fast achievement of the interpolation and map generation algorithms. Partially Supp. by DGAPA-UNAM.


Endogenous electric fields of active neurons are known to participate in neuronal synchronization. In 85 nA/m2 (KCl), in vitro hippocampal brain slices exhibit neuronal population burst-firing that shares physiological similarities with interictal epileptic spikes. We have recently demonstrated that such population activity could be paced with orthodic and amiodaronic stimulation of the tissue (*Neuroph. 73:287-89, 1993). We sought to determine whether such entrainment could be replicated nonsynchronically with electric fields. Transverse slices 400 μm thick were prepared from the hippocampus of 125-150 gm female Sprague-Dawley rats with an isoflurane-chloralose anesthetic and placed in an interface type perfusion chamber at 32-36°C. Autonous burst firing was induced with 85 nA/m2 (KCl) in the perforate. Pulsed dipole electric fields were created using 2 platinum plate electrodes placed 1 cm apart within the perfusion bath with a tissue slice in between. Extracellular recordings were performed with microtip electrode.

Twenty-two experimental trials were performed on 22 slices from 21 rats. Square wave electric field pulses were delivered in trains, 0.75-1.3 Hz, 0.3-1.0 msec duration, delivering brief steady state fields of 15-85 mV/m. In each experiment, by increasing the field strength, pulse duration, and, in the response of the neurons could be entrained into a 1:1 relationship with the field pulses. At higher intensities of pulse strength and duration, strict time locking of stimulus and response was seen. At pulse frequencies above the natural interburst interval of the tissue, more complex responses could be observed. These results demonstrate that a burst-firing autonomous neuronal network can be entrained with relatively small pulsed electric fields. These results may be useful in the control of such systems.
EPILEPSY:

Spike of cells: memory cells

A 16-channel silicon probe array was used to record field potentials during seizures in the hippocampus. Current source density (CSD) analysis was applied to eliminate the effect of volume conduction. In the CSD profiles the wave components (< 50 Hz) were analyzed. An amplitude vs. depth vector was constructed by collecting the peak amplitudes from each CSD channel. Sanborn's non-linear projection technique was utilized to visualize the spatial distribution of the amplitude vectors in their higher dimensional feature space. The multidimensional location of each amplitude vector was projected and plotted in three dimensions. This method enabled us to examine the quasi-trajectory of the epileptic afterdischarges. Two different states were observed in these plots: attractor state and a continuously changing state (CCS). In the attractor state the trajectory repeatedly changed within a subvolume of the feature space while in the CCS the consecutive amplitude vectors usually formed a curve changing toward a definite direction and showed certain linear tendencies. Typically, the initial CCS phase was followed by an attractor phase. This first attractor state, representing the main part of the afterdischarge, turned into a complex CCS. This complex state usually contained more than one CCS segment. Seizures were terminated in an elongated attractor phase. Traditional non-linear dynamical analysis methods were also performed (fractal dimension calculations on original and normalized signals and recurrence plots) and they indicated chaotic dynamics during the attractor phase and non-autonomous dynamics during the CCS. These methods are useful for the classification of spatially-temporally distinct patterns.

Epileptic field patterns and unit activity evoked by perforant path (PP) or commissural (COM) stimulation were compared to the CA1-dentate axis, CA3 and entorhinal regions using multisite silicon probes in freely moving rats. Both stimulation sites induced 2-4 Hz rhythmic afterdischarges with superimposed 100-200 Hz oscillations and 200-400 Hz population discharges. PP-induced seizures were initiated in the entorhinal cortex-dentate gyrus axis and later entrained the CA3-C1 regions, whereas COM stimulation triggered afterdischarges in CA3-C1 and entrained the entorhinal cortex-dentate gyrus. During the main part of the seizure all regions discharged synchronously without any apparent lead by any of the regions. Following bilateral removal of the entorhinal cortex the basic pattern of AD in the CA3-C1 axis remained similar. The primary afterdischarge always terminated first in the CA1 region, heralded by a slowly spreading (0.1 mm/sec), low amplitude, 70 Hz oscillation. Population burst initiation migrated within the CA3a-a axis. Non-synaptic population discharges of CA1 pyramidal cells were occasionally observed. Basket cells discharged primarily during the population spikes whereas other interneurons sustained long trains of fast (>200 Hz) spike trains. We therefore hypothesized that our seizure inducing systems exist in the intact brain: the CA3-C1A1 circuitry and the entorhinal-dentate network. These oscillating circuits are normally coupled, but they may also sustain afterdischarges independently.

DYE COUPLING OF NEURONS IN THE HIPPOCAMPUS IMPLIES A ROLE FOR GAP JUNCTIONS IN EPILEPSY. M. Penttonen, A. Bragin, A. Sik, and G. Buzsaki. CNBM, Rutgers University, Newark, NJ 07102

In awake rats hippocampal epileptic seizures first terminate in the CA1 region by a slowly spreading (0.1 mm/sec) (60-80 Hz) fast oscillation. A likely mechanism responsible for the oscillation is electrical excitation of pyramidal neurons from gap junctions (Bragin, et al; this meeting). In this experiment we tested this hypothesis by intracellular injection of biocyn or neurobiotin into pyramidal neurons in the CA1 subfield. During afterdischarges induced by 200 Hz stimulation of commissural pathways or by local microperfusion of ECI after dye injection and withdrawal of the pipette from the cell body. Intracellular stimulation of ECI injection induced a large intracellular depolarization coupled with extracellular DC shift. Rhythmic afterdischarges were initiated during recovery from depolarization. In intact rats < 3% of the injected neurons showed coupling. In rats with afterdischarges > 40% of the labeled pyramidal cells were dye coupled with other pyramidal cells, interneurons or astrocytes. The labeled pyramidal neurons were most prominent in the pyramidal layer. Electromicroscopic investigation of gap junctions between dye coupled cells was under way. We hypothesize that gap junction-mediated cell coupling and associated decrease of the cell resistance may be responsible for the termination of epileptic activity.

INTRINSIC RESPONSES AND MORPHOLOGICAL FEATURES OF NEURONS IN THE RAT PERIRHINAL CORTEX. R.A. Batty and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

Recent interest in the perirhinal cortex results from its involvement in memory, and its ability to rapidly develop kindled seizures with short latencies. The seizure data suggested direct connections between the perirhinal and motor systems. We confirmed this suggestion in anatomical studies. The layer V cells were project demenly to the frontal motor cortex. Although little was known about these cells, Beggs & Karten (Brain Res., 665:18-32, 1994) recently described several features of cortical cells that we observe in the perirhinal cortex. In the present study, intracellular recordings were made from over 60 perirhinal cells in a coronal slice preparation (McIntyre & Wong, J. Neurophysiol., 57:1305-1307, 1987). Intracellular recordings were studied with depolarizing and hyperpolarizing current injections. In general, the layer V cells were depolarized and hyperpolarizing current injected. Additionally, these dendritically-sparse layer III cells showed no anodal break response after hyperpolarizing current injection. Conversely, the large, layer V cells frequently displayed 'intrusive bursting' to depolarizing current injection at their resting potentials, which changed into single spike mode after intracellular injection. These dendritically-extensive layer V cells always showed a depolarizing anodal break response. It is these dendritically branching layer V cells that project to the motor cortex and may be responsible for projecting limbic discharges into their convulsive form.
RADIAL ARM MAZE PERFORMANCE AFTER PARTIAL HIPPOCAMPAL KINDLING. L. Sun, Lengue*, D. Brandreth, D. Young and B. Shon Dept. of Pharmacology, Clinical Neurological Sciences, University of Western Ontario, London, ON, N6A 5C5, Canada.

In previous studies (Behav Brain Res 40; 11, 1990: Hippocampus 4: 696; 1994), we found that rats that were previously trained on a radial arm maze (RAM) with external cues showed a 'retention' deficit for up to 25 days after partial kindling of the hippocampal CA1 (5-10 afterdischarges (ADs) over 3 days). The partially kindled rats were trained in the following 3 experiments: (1) to find the minimal number of ADAs required for RAM deficits; (2) to test if RAM deficits are also found in an internal cue maze; and (3) to test whether acquisition is affected in the partially kindled rats. In the first experiment, rats were trained on an external cue RAM with 4 arms baited, and rested for a week before partial kindling. In the 1st experiment, AD deficit, mainly in reference memory, was found in rats receiving 5-10 hippocampal ADs, and at 3 weeks only after 10 ADs. In the 2nd experiment, the rats were trained on an external cue maze and an internal cue maze before kindling. The internal cue maze had minimal extramaze cues, and the 4 baited arms with internal cues (e.g., carpet, soft paper, etc. on the floor) were shuffled before each trial. After partial kindling (15 ADs), rats showed deficits only on the external but not the internal cue maze, consistent with the literature on hippocampal lesion. In the 3rd experiment, rats were first trained on a RAM in one room, then kindled, and acquisition on the RAM with a different set of baited arms was conducted in a new room. There was no difference in the acquisition rate between the kindled and control rats. In conclusion, partial hippocampal kindling disrupted the performance of a previously learned external cue RAM but not that of an internal cue RAM. The acquisition of a new RAM does not seem to depend on whether rats are partially kindled or not. (Supported by NS 25383).


The anomalous sprouting of mossy fibers from dentate granule cells has been identified in human epilepsies and several seizure models. It is unknown whether mossy fiber sparring occurs following subcortical degeneration of the hippocampus (HIP), via transection of the fimbria-forx (PP). Previous research has demonstrated permanent changes in the excitatory neuronal activity, neurochemistry, and morphology in the HIP following FF lesions (Buzsaki et al., 1989; Lehtinen et al., 1993). In our first experiment, we attempted to map out the distribution of Tmmm granules in the supragranular cortex of the HIP and to plot the time-course of sprouting development following FF transection in rats. Mossy fiber sparring was first evident, only in the dorsal aspect of the HIP, at about 14 days post FF lesion. The distribution of Tmmm granules was increased in a continuous yet patchy pattern of granules being observed in the supragranular region between tips and crests of the dentate gyrus. The degree of sprouting did not appear to be greater at 30 days post lesion.

In a second experiment, we implanted bilateral electrodes into the perforant path in the primary cortical pathway into the HIP, 30 days following FF lesioning. Kindling stimulation was administered daily until generalized seizures were elicited. Afterkindling, rats were significantly lower in FF lesioned rats, but we found no significant differences in kindling rates between FF lesioned and non-lesioned rats. FF lesioned kindled rats exhibited a continuous and dense band of Tmmm granules that expanded into the inner molecular layer and was consistent throughout the entire HIP. Non-lesioned kindled rats demonstrated only moderate sprouting, similar to the degree observed with non-lesioned kindled rats. These results suggest that FF transections and perforant path kindling can independently induce mossy fiber sprouting, and in combination produce additive effects. Supported by MRC.

QUENCHING: INHIBITION OF DEVELOPMENT AND EXPRESSION OF AMYGDALA KINDLED SEIZURES WITH LOW FREQUENCY STIMULATION R.B. Wilson, Y.-L. L., B. Rogers, H. L., T. Heptner, R. M. Psychological Branch, NIMH, Bethesda, Md. 20892.

Using low frequency stimulation parameters, similar to those that induce long-term depression (LTD) or depolarization in vitro, we attempted to alter amygdala kindled seizures in vivo. Male Sprague-Dawley rats were implanted with electrodes in the left amygdala and connected to a 60Hz biphasic square wave pulse generator for 1 sec at their afterdischarge (AD) threshold. Immediately after the cessation of afterdischarge or seizure activity, the 'quenching' stimulation was administered at the same electrode for 15 minutes at a frequency of 1 Hz, using a 0.1 msec pulse width, and an intensity of 100µA over the AD threshold. Controls received either no current (sham-stimulation) or high frequency stimulation (100 Hz, 1 msec pulse width, 100µA AD threshold) for 15 minutes. All of the sham-(=n=4) and high-frequency stimulated (=n=4) animals kindled, developing increasing afterdischarges and seizures within 10-14 days of stimulation. In contrast, 7 of the 8 rats that received quenching stimulation did not exhibit afterdischarge or seizure progression, although all of these rats exhibited ADs on day 1 of stimulation. The afterdischarge thresholds of the kindled control groups decreased or stayed the same after kindling, while they markedly increased in the animals that were quenched. This effect persisted for several weeks after quenching was discontinued and kindling stimulation resumed.

In fully kindled animals, a week of quenching (without concurrent kindling) resulted in an increase in the seizures (100/sec) and a long lasting decrease in some animals) decrease in the seizure response to the original kindling stimuli. This did not occur in animals given sham stimulation for one week.

These findings suggest that rapid kindling treatment using low frequency has profound effects on amygdala kindled seizure development, expression and thresholds. Whether quenching is mechanically related to either LTD or depression requires further study. The marked and long-lasting effects of quenching suggest possible clinical applications not only for epilepsy, but also for other neuropsychiatric disorders where threshold phenomena may be crucial to illness emergence.
EPILEPSY: kindled animals and patients. The hippocampal structure in rats has been studied extensively. In this study, we have used the RGDs tetrapeptide to examine a potential role for integrins in an animal model of epileptogenesis in vitro. Kindling was induced by stimulation of the electrically stimulated membrane. The RGDs tetrapeptide was used to examine the effect of integrin-ECM interactions on epileptogenesis. The results indicate that the presence of the competitive peptide, hippocampal slices become hypertrophied over time, indicating that interfering with ECM-integrin binding has an effect on neuronal excitability. The disruption of the ECM-integrin interaction appears to increase neuronal excitability, these results suggest that integrin may participate in neuronal signaling through RGD binding sites.

Work supported by NINDS NS27903.


This study examined whether electrically stimulating different nuclei in the amygdala would produce changes in afterdischarge threshold (AD) and seizure development. Male rats were implanted with electrodes aimed at the basomedial (BL) and central (CA) and the amygdaloïd (Amy) zone. The animals were stimulated four times daily (40 Hz, 30 sec). The animals were killed at 7 days, and hippocampal sections were stained with hematoxylin and eosin. The results indicate that the presence of the competitive peptide, hippocampal slices become hypertrophied over time, indicating that interfering with ECM-integrin binding has an effect on neuronal excitability. The disruption of the ECM-integrin interaction appears to increase neuronal excitability, these results suggest that integrin may participate in neuronal signaling through RGD binding sites.

Work supported by NINDS NS27903.

EPILEPTIC KINDLING IS ASSOCIATED WITH A LASTING INCREASE IN THE EXTERNAL LEVELS OF KYNURENE ACID IN THE RAT HIPPOCAMPUS. P. Schwanitz, A. P. Wu, T. Monna and Z. Vezzani. Maryland Psychiatric Research Center, Baltimore, MD 21228 and Mario Negri Institute for Pharmacological Research Laboratory of Neuropharmacology, Milano, Italy.

The extracellular levels of kynurenic acid (KYN), an endogenous broad spectrum excitatory amino acid receptor antagonist with anti-neurotoxic and antiinflammatory activity, were assessed by microdialysis in the hippocampus of kindled rats. One week after the completion of amygdala or hippocampal kindling (stage 5: occurrence of tonic-clonic convulsions), the extracellular concentration of KYN was 1.7±0.1-fold higher than in sham-operated controls (P<0.001). This effect was observed to the same extent in the hippocampus ipsilateral and contralateral to the electrical stimulation. Moreover, whereas 50 μM verapamil, applied through the probe, reduced extracellular KYN by 28% within 1 h in control rats (P<0.001), verapamil was ineffective in stage 5 kindled rats. During the initial phases of hippocampal kindling (stage 2: stereotropies, retraction of a forelimb), extracellular KYN levels and the effect of verapamil were similar to controls. The activity of KYN's biosynthetic enzyme, kynurenine aminotransferase, which is preferentially localized in astroglia, did not change in the hippocampus one week after stage 5 seizures. These data indicate an enhanced liberation of KYN from hippocampal astrocytes due to an impairment of its normal regulatory mechanism in fully kindled animals. This may be of relevance for the control of excitatory amino acid receptor function during epileptogenesis.

Supported by USPHS grant NS16102.

ROLE OF NERVE GROWTH FACTOR IN KINDLING AND KINDLING-INDUCED MOSSY FIBER SPRouting. M. N. Masatoshi, J. N. A. King, P. C. Rashid, C. E. M. van der Zee, J. Diamond, M. F. van den Berkle, J. R. Racine. Department of Biomedical Sciences and Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8N 3X3.

Repeated subconvulsive electrical stimulation of certain areas of the forebrain leads to kindling, a progressive and permanent amplification of evoked epileptiform activity which is a model for human temporal lobe epilepsy. Kindling induces synthesis of nerve growth factor (NGF) protein and NGF mRNA, increases mossy fiber sprouting and functional synaptic integration have been observed in the hippocampi of kindled animals. We have shown that intraventricular administration of antibodies and peptide antagonists of NGF retard amygdaloid kindling and block kindling-induced mossy fiber sprouting. To determine whether NGF can enhance the development of kindling and kindling-associated sprouting, rats receiving intraventricular NGF or saline were killed in the amygdala by twofold intraventricular injections. Rats receiving intraventricular NGF received fewer (6.7±0.9) stimulations to reach the fully kindled state than rats receiving saline (13.7±2.9). Following kindling, spraying the mossy fibers into the stratum oriens of the CA1 region of the hippocampus is measured by Timm staining, and kindling-induced hippocampal damage is assessed by performing total cell counts for the hilus. Timm staining and cell counting analyses are in progress and preliminary data will be reported. Our findings further demonstrate the role of NGF in kindling.

EFFECT OF KINDLING ON CALCIUM CURRENTS IN ADRENALECTOMIZED AND ADRENALLY INTACT RATS. H. Kariya, A. B. Miller, E. F. De Kloet and M. Jodar. Takeda Research Institute, 2300 RA Leiden, 2506, Exp. Zoology, Univ. of Amsterdam, 1098 SM Amsterdam, The Netherlands.

Stress is known to affect the incidence and nature of seizures in epileptic patients. To study the effect of corticosteroids on epilepsy we used an animal model for temporal lobe epilepsy, i.e. kindling. Via electrodes implanted in the hippocampal Schaffer collaterals, male Wistar rats were tetanized twice daily with 50 Hz frequency stimulation for two seconds with a current intensity of 200 μA. After the tenth class 5 seizure according to the scale of Racine, the rats were fully kindled. Three to four weeks later rats were either adrenalectomized or sham operated. Five to seven days later the animals were sacrificed. Four experimental groups were investigated: non-kindled sham operated, kindled sham operated, non-kindled ADX and kindled ADX rats. Of these groups a number of parameters were studied, including the calcium currents in CA1 neurons, the in-situ patch-clamp technique in the slice. Kindling and ADX increased the amplitude of low threshold calcium currents (LTCC). Voltage properties and kinetics of LTCC were not affected. Adrenalectomy of the kindled rats however did not further increase the LTCC currents, indicating that ADX is not acting as a subthreshold current modulator. In contrast to the LTCC-currents, kindling caused a decrease of the high threshold calcium (HTCC) currents. Adrenalectomy did not affect these HTCC-currents, neither in the non-kindled nor in the kindled rats. The expression of the voltage-gated L-type Ca-channel subunits and corticosteroid receptors in the kindled and ADX rats is presently under investigation. Previous work reported that LTCC-currents are mainly located in the dendrites of pyramidal CA1 neurons. HTCC-currents can be found near the soma. We tentatively conclude that both kindling and ADX particularly increase the number of Ca-channel region, an area where also a considerable part of the sympathically induced Ca-influx takes place.
T73.1


Changes in the expression of nicotinic acetylcholine receptor (nAChR) have been among the first reported neurochemical landmarks of Alzheimer’s disease (AD). The availability of nAChR subunit specific assays as a probe has allowed to investigate the subunit gene expression and its relation to pathology intracellular changes. In situ hybridizations were performed on autopsy tissues of the superior frontal gyri of AD brains (n=56) and age-matched controls (n=55), using a digoxigenin-labeled a4 nAChR subunit antisense cRNA probe. Hybrids were visualized by an alkaline phosphatase-scoupled digoxigenin antibody. Simultaneous detection of tau-protein or glial fibrillary acidic protein was achieved by indirect immunoperoxidase technique. As reported earlier, a4 mRNA was detected in many neurons of all cortical layers. We did not observe general changes in the distribution of a4 subunit nAChR expressing neurons in AD as compared to controls. Corresponding in the density of structures expressing tau protein, the number of a4 mRNA-containing spines decreased in AD, especially in the pyramidal cells of layer III/IV. Neurons that were heavily labeled for the tau-protein expressed little or no a4 mRNA.

The present results underline the importance of the relation between the expression of pathologically modified filaments and nAChR mRNA. Further studies at the EM level on the mRNA distribution of a4 and other subunits will provide additional information on nAChR mRNA transport under normal and pathologic conditions.

Supported by the Deutsche Forschungsgemeinschaft, grant Schr 283/6-2

T73.2

HCNP gene expression is decreased in the hippocampus of postmortem human brain from patients with senile dementia of the Alzheimer type. N. Matsukawa1, H. Okaeda2, N. Tatedo1, and K. Oka1. 12nd Department of Int. Med. 3rd Department of Molecular Biology, Nagoya City Univ. Mizuho-ku, Nagoya, 467-2: Department of Molecular Biology, Noryori-Fukiushuma Hospital, Noryori-cho, Toyohashi, 440: 4Discovery Research Laboratories, & Sumitomo Pharmaceutical Research Center, Konohana, Osaka, Japan.

We previously demonstrated that hippocampal Cholinergic Neurotransmission-Stimulating Peptide (HCNP), a novel peptide purified from 10-12 day old rat hippocampus, specifically enhances acetylcholine synthesis in medial septal nuclei in vitro. The septo-hippocampal system plays an important structural role in memory formation. Moreover, it is known that certain pathological changes, such as neuron loss, glialosis, plaques and tangles, are found in the hippocampus of patients with Alzheimer type dementia. In this study, we prepared total RNA from hippocampus and frontal lobe of postmortem human brain by the AGP method, mRNA was quantified by Northern blot analysis using cDNA specific for HCNP precursor protein as a probe. The results suggested that expression of HCNP precursor mRNA in hippocampus decreased in 5 cases with Alzheimer type dementia compared to 5 controls of normal aging, even after correction for the expression of β-actin mRNA. This result supports our previous findings documenting the lack of neurotrophic substances in brains of patients with Alzheimer's disease. (Ojika,K. et al., Banbury report 15,Cold spring Harbor,NY:1983:285-295).

T73.3

FUNCTIONAL REGULATION OF NEOCORTICAL PYRAMIDAL NEURONES BY BOTH MUSCARINIC AND NICOTINIC RECEPTORS. P. Cheesell, M.A. Simmonds1, and P.P. Humphrey. Glaxo Institute of Applied Pharmacology, Cambridge, CB2 5DH, and 1Dept. Pharmacology, School of Pharmacy, London, WC1N 1AX, UK.

A novel cortical brain slice preparation for use with extracellular "grease-gap" recording techniques has been developed, allowing study of both glutamatergic and cholinergic responses of neocortical pyramidal neurons which form the transition through the neocortical pathway. Concentration-effect curves to the muscarinic/nicotinic agonist carbamol and the nicotinic agonist DMPP yielded mean EC50 values of 29.5 and 13.2 μM, respectively. Carbamol-induced responses were abolished by atropine (0.2 μM) and mecamylamine (10 μM). This antagonism was not competitive. Conversely, concentration-effect curves to DMPP were shifted in a parallel rightward manner by mecamylamine, and were unaffected by atropine. Depolarization responses to glutamate were reversibly antagonized by D-AP5 (30μM) and CNQX (15 μM), these antagonists had no effect on carbamol- or DMPP-induced depolarizations.

This preparation allows reproducible quantification of depolarization responses of pyramidal neurons whose axons pass through the corpus callosum to contralateral cortex; such studies indicate that intracellular and functional regulation of the activity of these neurones by nicotinic and muscarinic receptors present on their cell bodies and dendrites. These same neurones degenerate in Alzheimer’s disease, culminating in marked cortical glutamatergic hypoactivity, which underlies the cognitive deficits observed. We propose, therefore, that therapies aimed at activating nicotinic as well as muscarinic receptors are more likely to succeed in ameliorating the cognitive symptoms of the disease than those aimed at muscarinic activation alone.

T73.4


The vesamicol binding site on the cholinergic synaptic vesicle is a novel target for the development of radiopharmaceuticals for imaging cholinergic innervation. Previous studies using [3H]vesamicol have demonstrated marginal reductions in the densities of vesamicol binding sites in frontal cortex and increased densities in the temporal cortex in Alzheimer’s disease (AD). Vesamicol binding site densities correlated poorly with the loss of choline acetyltransferase (ChAT) activity in AD. These conflicting findings suggest that [3H]vesamicol may label also non-cholinergic sites in the cerebral cortex. In the present study, we have evaluated the novel vesamicol analog [125I]p(o)-MBT as a probe to assess cholinergic synaptic integrity in the temporal cortex of AD and neurologically normal age-matched control subjects. Autoradiography binding analysis using aminobenzoxazenomiacil (ABV) to define non-specific binding, revealed a high affinity binding site with a Kd value of 3.2 ± 1.2 nM in the temporal cortex in aged control subjects. Similar affinity values were observed for [125I]p(o)-MBT binding in AD. The density of [125I]p(o)-MBT binding was reduced significantly in AD temporal cortex (Bmax=11.6 ± 2.8 pg/mg) as compared to control values (Bmax=19.5 ± 3.2 pg/mg). The decrease in [125I]p(o)-MBT binding was correlated with ChAT activities (r=0.88) in AD temporal cortex. These results suggest that [125I]p(o)-MBT may be useful for assessing in vivo the loss of cholinergic projections with PECT in AD and other dementing disorders.

T73.5


Alzheimer’s disease currently affects approximately 4 million people in the United States, with 100,000 new cases being reported each year. This disorder is typified by several neurological and psychiatric symptoms, including memory loss and confusion. In the following series of studies we have expanded on these results in glucocorticoid-treated animals following ICV injections of the ACh neurotoxin, AF64A (Inovent/venkis), or intrahypothalamic injections of the ACh toxin, saporin. In experiment 1, four groups of male rats were used: 1) ICV injections of AF64A in animals treated for 7 days with corticosterone (CORT, 7 mg), 2) ICV injection of vehicle + CORT, 3) ICV injections of AF64A + 7 days of saline injection (CONT), and 4) ICV injections of vehicle + CONT. In experiment 2 the following groups were used: 1) bilateral injections of saporin into the nucleus basalis (NB), 2) bilateral injections of vehicle into the NB (CONT), and unilateral injections of saporin into the nucleus basalis (MA), and 4) unilateral injections of vehicle into the MSA. Approximately 14 days following surgery, all animals were assessed for retention of an eight-trial Water Maze. Our results reveal a significant impairment in a variety of behavioral parameters associated with spatial learning in the rat following injections of saporin into the NB and the MSA. In support of previous studies observed in the MSA animals. In addition, a significant potentiation of AF64A on spatial learning was observed in glucocorticoid-treated animals. Our results strongly support the use of selective ACh neurotoxins as a model of Alzheimer’s disease. Other studies have suggested that cholinergic stress is involved in the pathogenesis of Alzheimer’s disease.
773.7 MUSCARIC ANAGONISTS INCREASE HIPPOCAMPAL PHOSPHATIDYL INOSITOL TURNOVER IN VIVO AND ATTENUATE HEMICHLONIUM-3-INDUCED AMNESIA IN MICE. K.M. West, D.S. Chaps, I.T. Nowakowski, I.T. Forman, D.M. Nason, A. Villahermosa and D.R. Lipton*. Pfizer Inc., Central Research Division, Department of Neuroscience, Groton, CT 06340.

Alzheimer's disease (AD) results in a loss of the cholinergic projections important in learning and memory that interact directly with post- synaptic m1 receptors, which are preserved in AD, may provide effective palliative therapy. We have examined the in vivo activity of three potent muscarinic agonists (pilocarpine, LY-247608 and SDZ-ENS-163) using biochemical (in vivo phosphatidylinositol turnover) and behavioral (passive avoidance) models of central cholinergic function. For in vivo pilocarpine, 104-micrograms was injected i.e.c. (2 microL) 24 hr prior to test compounds to prelabel the hippocampal phospholipid pool. Lithium (10 mg/kg s.c.) was administered 3 hr prior to tissue sampling and the test compounds were administered s.c. 1 hr prior to tissue sampling. The accumulation of inositol phosphates (IP3) in the hippocampus was determined following extraction and ion exchange chromatography. Significant elevation of hippocampal IP3 was produced by pilocarpine at 10-32 mg/kg s.c. by LY-247608 at 3.45 mg/kg s.c. and by SDZ-ENS-163 at 5.25 mg/kg s.c. A one- step through passage assay (PA) procedure was utilized to test the ability of these compounds to reverse hemicliromium-3 (HC-3) induced amnesia. Test step-through latency was reduced by 50-60% following treatment with HC-3 (1.78 mg i.c.v.) 2 hours prior to the training session. Pilocarpine (1.78-10 mg/kg), LY-247608 (1.10 mg/kg) and SDZ-ENS-163 (1.78-17.8 mg/kg) attenuated HC-3 induced amnesia. Most notably, the effective dose range of LY-247608 and SDZ-ENS-163 in PA spanned a range of 50 mg/kg, in contrast to our experience with AChE inhibitors, which show a more narrow range of effective doses in this model. We conclude that selective muscarinic agonists may be useful in treating demented states such as AD where the central cholinergic system is compromised.


The cholinergic hypothesis of AD has influenced much of our thinking on learning and memory over the last decade. However, cholinomimetic agents have had limited success in the treatment of AD dementia. The noradrenergic system has also been implicated in cognitive processes and may be depleted in AD. This study investigated the efficacy of a novel AChE inhibitor and noradrenergic agonist (SDZ-ENS-163, next abstract) have shown 32-antagonists potentiate the ability of AChEIs to enhance long-term memory retention. We describe studies in which we examine several in vivo parameters to assess the efficacy of a novel AChE inhibitor and noradrenergic properties (next abstract, Varga et al, 1995). PI1467 dose-dependently inhibited rat striatal AChE activity (ID50=3.1 mg/kg, i.p.), this effect was reversible. In microdialysis studies, the compound increased ACh levels in the rat hippocampus 4-6 fold following a dose of 5 mg/kg i.p., an effect shared with other AChEs. At 1.25 - 5 mg/kg i.p., PI1467 significantly increased hippocampal NE release (similarly to d-tubocurarine) in a dose-dependent manner, a characteristic not shared with other AChEs. Doses of PI1467 which elevated ACh in NE release also enhanced long-term memory retention in a step-down passive avoidance paradigm in rats. Functional assays showed that PI1467 competitively antagonized pre- and postsynaptic A2-adrenoceptors in vitro and in vivo. These findings indicate that PI1467 elevates central ACh and NE levels via inhibition of AChE activity and antagonism of A2-adrenoceptor function.


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Pathological findings have indicated that cholinergic dysfunction of the brain in Alzheimer's patients appears to be compromised. In the clinic, some patients have shown improvements or delayed progression of symptoms during treatment with acetylcholinesterase (AChE) inhibitors such as tacrine. Here we report on the effect of CI-1002, a potent m2 adrenergic antagonist, CI-1002, and its ability to alter neurotransmitter release in two animal species. Adult male Long-Evans rats had microdialysis probes surgically implanted into the lateral prefrontal cortex and were allowed to recover for 24hrs. Saimiri sciureus, were chair-restrained and a probe placed in the putamen through a previously implanted burr-hole was adminisitered at 0.32, 1.0 or 3 mg/kg in each dose in saline at an hourly rate over 2 hours to control. This study demonstrates the potential of CI-1002 to elevate cholinergic tone in rodent and non-human primate species.

773.12 DETERMINATION OF BLOOD AND BRAIN ACETYLCHOLINESTERASE (AChE) INHIBITION AFTER ORAL DOSING OF CI-1002. M.Z. Emmerting, M.C. Calhoun, H. Leveque, E. Face*, W. Leppard, A.C. Baly, Parke-Davis Pharmaceutical Research Division of Warner-Lambert, 2800 Plymouth Rd., Ann Arbor, MI 48105. CI-1002 is a combined AChE inhibitor and muscarinic antagonist (Acetylcholinesterase, 1994, 59, 93). AChE inhibition in blood and different brain regions produced after oral dosing of 2 and 10 mg/kg doses of CI-1002 was determined by two different radiometric AChE assays. Doses of 2 mg/kg dose was decreased by 40 % compared to control at 0.5 and 2 hr and similarly to d-tubocurarine in a dose-dependent manner, while the other doses remained significantly lower than control over the next 6 hr. In brain, the enzyme activity in hippocampus was significantly reduced by 30% at 1 hr and 2 mg/kg dose of CI-1002 and by 54% at 10 mg/kg dose of CI-1002. A significant inhibition of AChE activity was also evident in the frontal cortex at 1 and 2 hr after the 10 mg/kg dose. Although lower levels of AChE activity were detected, no significant changes in the AChE activity of striatum or corpus striatum relative to control was detected at any dose or time point tested. These results showed that CI-1002 enters the brain in a sufficient quantity to cause inhibition of AChE activity. The level of AChE inhibition in brain is dose-dependent and correlates with the level of AChE inhibition detected in blood. Brain regions are differentially affected by CI-1002 with the greatest sensitivity being detected in the hippocampus. Finally, results will be presented to show that the level of AChE inhibition detected can be used to estimate the concentration of CI-1002 in tissues with reasonable accuracy.
773.13

BUTYRYLCHOLINESTERASE IMMUNOCHEMISTRY IN HUMAN CEREBRAL CORTEX. M.M. Messelmann*, C. Gesta, S. Pringsho, J.E. Smiley, 1Northwestern University, Chicago, IL; 2Harvard Medical School, Boston, MA; 3Mayo Foundation, Rochester, MN.

Plaques and tangles in Alzheimer's disease (AD) express butyrylcholinesterase (BChE) enzyme activity. This activity could conceivably come from degenerated remnants of premortally BChE-positive neurons. To test this hypothesis, we used monoclonal antibodies to detect BChE in sectioned human cerebral cortex. There were very few BChE-positive perikarya in non-demented young and aged brains. Most were located in the gyrual white matter or in layer 6 and were non-pyramidal, Limbic areas, especially the stratum oriens of the hippocampus, contained the highest number. The density of these neurons was 1-2 orders of magnitude less than that of BChE-positive tangles seen in AD. No immunopositive cortical axons were seen to account for the BChE-positivity of neuronal plaques in AD. Concurrent visualization of BChE immunoreactivity with BChE enzyme activity revealed distinctly double-labeled perikarya. These results support our conclusion that the plaque- and tangle-bound BChE in AD is unlikely to be of neuronal origin. In many of our specimens, neuritis appeared intensely BChE-immunopositive. Since neuritis are prone to non-specific immunostaining, this observation needs to be interpreted cautiously. However, the BChE-immunopositivity of neuritis is consistent with our previous expectations and our hypothesis that the plaque- and tangle-bound BChE is of neuronal rather than neuronal origin. The role of neuronal BChE remains mysterious. Diverse trophic functions have been attributed to BChE and may underlie its role in neuritis.

773.15

PHENESERINE, A NEW DRUG FOR ALZHEIMER'S DISEASE: FAVORABLE TOXICITY PROFILE. N.H. Greig, D.K. Ingram, Y.F. Lin, A. Brown, H.W. Holloway, T.T. Soncornt*. Laboratory of Neurosciences and Molecular Physiology and Genetics Section, NIA, NIH and School of Pharmacy, University of North Carolina, Chapel Hill.

Phenserine, a phephosphate of (+)-phystostigmine, is a novel, long-acting (t1/2=8 h), rapidly cleared (t1/2=10 min), brain-targeted (10-fold), and acetyl-selective (>50 fold) cholinesterase inhibitor. In rats, it significantly increases brain acetylcholine and robustly enhances cognition [NeuroReport 6: 481-4, 1995; Med. Res. Rev. 15: 3-31, 1995]. Phenserine is dramatically less toxic than phystostigmine (MDT >15 vs. 0.6 mg/kg, ip, rat). In initial toxicological studies, phenserine was administered once daily to rats at doses of 1 and 5 mg/kg ip (cumulative doses of 28 and 140 mg/kg) and was compared to saline-treated animals. Extensive evaluation of blood hemalogic and clinical parameters, including markers of renal and hepatic function, together with histological analysis of brain, kidney and liver, demonstrated lack of toxicity at doses more than five times greater than those which maximally enhance cognition. A standard pharmacologic screen revealed no evidence of direct action at neurotransmitter receptors and lack of autonomic activity in mice. Phenserine's optimal pharmacological properties, combined with its favorable toxicology profile in pre-IND studies, predict efficacy in upcoming clinical trials superior to that of other agents developed for Alzheimer's disease.

773.17


Apolipoprotein E (apoE) is implicated in cholesterol and phospholipid transport, particularly in the central nervous system where other apolipoproteins such as apoA1 and apoE are absent. ApoE is a polymorphic protein of which the major allelic forms are apoE2, apoE3 and apoE4. The latter is associated with familial and sporadic forms of Alzheimer's disease (AD). The number of apoE4 gene copies affects the levels of platelets and choline acetyltransferase (ChAT) activity in the hippocampus of AD patients (Poirier et al., Lancet, 1993, 342:697-9; Schmechel et al., 1993, PNAS 90:6949-53; Poirier, 1994, TINS 17:252-5). To further characterize the impact of the apoE4 allele(s) on cholinergic deficits in AD, we examined the effect of the number of apoE4 allele copy number on hippocampal and mesencephalic M1 and M2 receptor sub-types in the hippocampus and temporal cortex of post-mortem brains from AD and control apoE4 alleles with different apoE genotypes. The apoE4 allele copy number shows an inverse relationship with residual ChAT and micoacetylcholine binding sites in AD. Such a relationship was not seen in control patients. AD individuals lacking apoE4 allele copy number have significantly higher ChAT activity and receptor levels levels than control values. ApoE4 allele copy number did not correlate with apparent changes in M1 or M2 binding sites in AD. Finally, the effect of apoE4 allele copy number on cholinergic deficit response was examined in AD patients treated for six months with the acetylcholinesterase inhibitor, Tacrine. Data revealed that only 90% of the patients who do not possess the ApoE4 allele showed significant improvements following this therapy while 60% of the patients with at least one copy of the apoE4 gene did not improve, and even deteriorated, during the treatment. These data support the hypothesis that apoE4 plays an active role in cholinergic dysfunctions associated with AD. Supported by MRC.
**T74.1**

**SIGNIFICANT CHANGES IN THE HUMAN BRAIN HISTAMINERGIC SYSTEM IN ALZHEIMER'S DISEASE.**


Histamine is one of the neuropeptides present in subcortical projection systems. Neurofibrillary tangles are present in histaminergic neurons in Alzheimer's disease, but these areas have not been examined. We combined sensitive immunohistochemical methods with HPLC fluorometry to reveal the changes in brain histamine in Alzheimer's disease.

In normal human brain (n=5, mean age 82 yrs), histamine concentrations were highest in the hypothalamus, followed by substantia nigra, putamen, n. caudatus and different cortical areas. In Alzheimer's disease (n=9, mean age 81.5 yrs) histamine content was significantly reduced only in the hypothalamus (57%), temporal cortex (46%) and hippocampus (57%). The results reveal only neuronal storage sites of histamine in normal brain and in brain affected by Alzheimer's disease. In normal brains, dense networks of histamine-containing fibers were seen in the entorhinal cortex and subcortical. Moderately dense networks innervated hippocampal fields CA1-4 and dentate gyrus of the hippocampus were seen in the Embrie. The results reveal an extensive histaminergic system in human temporal lobe, and significant changes in this system in Alzheimer's disease.

**T74.3**

**EFFECTS OF THE RELEASE ENHANCER DuP 996 ON THE IN VIVO RELEASE OF DA AND 5-HT IN RAT STRIATUM.**

T. Clarke, D.R. Bryce and H. Rolls*.

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DuP 996 (Linopidine) is a release enhancer which possibly acts via interaction with a K-channel. It is now well established that in vitro, DuP 996 increases the K+-stimulated release of ACV, DA and 5-HT from slices, while microdialysis studies have shown that DuP 996 also increases in vivo ACV release. To determine the effects of DuP 996 on in vivo DA and 5-HT release, we measured extracellular DA and 5-HT levels by microdialysis in rat striatum.

Of the doses of DuP 996 tested (0.3 - 10 mg/kg) only 3.2 mg/kg produced a small (20%) increase in extracellular DA and 5-HT levels in rat striatum. High doses of DuP 996 (32 mg/kg) produced a more pronounced elevation (40-60%) in DA and 5-HT release, but extracellular levels of the metabolites, DOPAC, HVA and 5-HIAA were increased to the same extent and all animals developed severe disorders. The neurochemical effects of high doses of DuP 996 could therefore be related to toxic events.

In view of the marked effects of DuP 996 on evoked DA release in vitro, we also studied its effects on extracellular DA levels during K+ stimulation. To that end, 60 min after the perfusion for 60 min and before the maximal increase in DA release was measured (‘51’). Three hours later the effects of a second KCl perfusion on DA release were assessed (‘52’), after pre- and post-perfusion with 10 mg/kg DuP 996 or vehicle. Comparison of the ‘52’/‘51’ ratios for vehicle and DuP 996 treated animals showed that the K+-stimulated DA release was slightly, but not significantly, increased by DuP 996.

In these in vivo results confirm that DuP 996 does not enhance the basal release of DA and 5-HT, but do not corroborate the pronounced effects of DuP 996 on evoked DA release in vitro.

**T74.5**

**THE DORSAL RAPHE AND DEPRESSION IN ALZHEIMER'S DISEASE.**


There is considerable evidence supporting a role of serotonergic systems as well as noradrenergic systems in the pathophysiology of major depression. We have previously demonstrated an association between major depression complicating Alzheimer’s disease (AD) and disproportionate neuronal loss within the noradrenergic locus coeruleus (Soc Neurosci Abstr 1992;18:206). In order to investigate the possible association of serotonergic pathology and depression, we used an anti-5-hydroxytryptamine antibodies from rabbit (PHB) (gift from RGH Cotton, Melbourne) to selectively label serotonergic neurons within the dorsal raphe (DR) nucleus of 5 patients with AD complicated by major depression (preliminary assessments based upon prospectively collected psychiatric evaluations) and from 8 age-matched patients without this complication. Sections (12 µm thick) of DR 1.2 mm and 1.68 mm caudal to the oculomotor nucleus were selected from serially sectioned formalin fixed brainstem (transverse plane). Numbers of PHB-immunoreactive neuronal profiles greater than 12.5 µm in maximum diameter within defined boundaries of the DR were counted manually (40X). Mean profile counts tended to be nonsignificantly higher in depressed than in non-depressed patients at both anatomical levels evaluated: 177.8 ± 57.3 and 204.3 ± 61.4 in depressed patients versus 175.1 ± 61.3 in patients with depression at rostral and caudal levels. Thus, there does not appear to be a relationship between loss of serotonergic DR neurons and depression in AD.
Vulnerability of pyramidal neurons immunoreactive for an N-
acetylated dipetide in Alzheimer’s and Huntington’s disease.
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Zoologisches Institut**, Universitat Karlsruhe, 76128 Karlsruhe,
FRG.
An increasing body of evidence suggests a neurocommunicative role for N-
acetyl-asparagine-glutamate (NAAG), especially in putative glutamatergic
pathways. NAAG pathways in central cortex of primates and NAAG is
preferentially localized to a subpopulation of pyramidal neurons.
Subsets of pyramidal neurons associated with corticostriatal, corticothalamic
and corticopontine projections have previously been described to exhibit
NAAG-like immunoreactivity (NAAG-LI) in human brain (Passant et al.,
1994). In the present study we investigated the functional impact of
neurodegenerative disease on this type of NAAG-LI neuron. One characteristic
feature of Huntington’s disease (HD) and Alzheimer’s disease (AD) is the
selective degeneration of subsets of glutamatergic pyramidal neurons. In
HD, lesions are most prevalent in layer III and VI of frontal lobe and primary
visual cortex and in layer IVc of the temporal lobe. In AD, lesions occur especially
in layers III and V of the neocortex, and in the CA1 field and subiculum of
the hippocampus. Our preliminary results suggest selective sparing of
subpopulations of NAAG-LI pyramidal neurons in frontal cortex and primary
visual cortex of HD post mortem brain. In both regions NAAG levels (determined
by an anti-NAAG antibody) and NAAG-LI pyramidal neurons (layer
III, 1Vh, VI) are not significantly altered compared to control. Glutamate
levels on the other hand decreased up to 70%, suggesting either loss of
glutamatergic neurons or metabolic alterations. In AD, NAAG levels are
unaltered in hippocampus and in frontal, primary visual and temporal cortex.
These findings suggest different degrees of vulnerability for glutamatergic
pyramidal neurons depending on the presence or absence of NAAG.
T4.13
CALCIUM-U-LIKE PHOSPHATASE ACTIVITY IN CONTROL AND ALZHEIMER'S DISEASE STRIATE CORTEX. C.J. Laster and J.M. Lee. Neurosciences Program, Department of Pharmacology and Toxicology, Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153
Neurofibriillary tangles found in brains of individuals with Alzheimer's disease (AD) are composed of hyperphosphorylated forms of tau protein. The phosphorylation state of proteins is determined by the balance of kinase and phosphatase activity. Increased tau phosphorylation may result from an increase in kinase activity and/or a decrease in phosphatase activity. The present study examined calcium-u-like phosphatase activity in homogenates of postmortem striatum cortices from age-matched normal (ADN) and AD subjects (N=7). The homogenates were separated into particulate and cytosolic fractions. Phosphatase activity was assessed using the substrate pyrophosphate-phosphatase (10 mM pyrophosphate measured as a change in absorbance at 405 nm) due to the formation of p-nitrophenol. Measurements of phosphatase activity were determined in the presence or absence of calcium u-like phosphatase activators (1 and 0.1 mM CaCl_2 and 0.5 mM NiCl_2, respectively). Calcium u-like phosphatase activity was observed in the presence of nickel. The nickel-stimulated increase in phosphatase activity was antagonized by trifluoperazine and cyclopiazon A. These biochemical properties of the particulate phosphatase activity are consistent with calcineurin. As a percentage of basal, nickel produced a 65% increase in phosphatase activity in control particulate fractions, but only a 24% increase in AD samples (p < 0.05, student's t-test). Absolute values of basal activity did not significantly differ between control and AD samples. In the cytosolic fraction, no stimulation of phosphatase activity by divalent cations or inhibition by TPP and CaA was observed. In addition, no differences between control and AD cytosolic findings suggest that calcineurin-like activity may be reduced in striate cortex in AD. Current studies are examining the relationship between calcineurin activity with changes in neurodegeneration.

T4.14
The familial Alzheimer's disease-causing amyloid precursor protein (APP) 670/671 double mutation (APP 670/671) is known to give a 3-fold increase of β-amyloid production in cultured fibroblasts from affected individuals. Since APP processing can be regulated by protein kinase C (PKC) activation, we investigated whether the phenotype of the APP 670/671 mutation included a PCK activity in the primary skin fibroblasts. PKC activities and levels were studied in the particulate and soluble fractions from 6 mutation bearing and 7 control cell lines. PKC activity in the soluble fraction, determined as the phosphorylation of endogenous histone substrate, showed an age-related change (r=0.569, p<0.05, Fisher's test) in both cases. The correlation coefficient in the particulate fraction was 0.474 (p=0.06). The average PKC activity was 1.7 and 2.7 pmol/mg protein respectively. In the particulate fractions were below the assay detection limit.

T4.15
AGING CAUSES SELECTIVE LOSS OF CALBINDIN-D_28K FROM THE CHOLEINERGIC NERVOUS SYSTEM OF THE HUMAN BASAL FOREBRAIN.植被* and M. Genua. Harvard Medical School, Boston, MA 02215 and Northwestern University Medical School, Chicago, IL 60611.
Age-associated changes in calbindin-D_28K immunoreactivity within the cholinergic neurons of the basal forebrain (BFCNs) were studied in the human brain and compared to changes in the cholinergic neurons that are involved in the increase in growth factor receptor (NGF) immunoreactivity. Calbindin-D_28K, NGF and NGF immunoreactivity were visualized immunohistochemically in adjacent sections of the basal forebrain in 12 brains from normal individuals 20-91 years old. The BFCNs in the majority of individuals over 70 years (n=6 of 7) displayed a marked loss of calbindin-D_28K immunoreactivity when compared with individuals younger than 70 years of age (n=5). This loss was observed in all BFCN groups and reached a maximum of 80% at 85 years of age. The selectivity of this observation. Loss of calbindin-D_28K would deprive cholinergic neurons of the capacity to buffer intracellular calcium levels and leave them vulnerable to processes that increase intracellular calcium and lead to neuronal death. The age-related loss of calbindin-D_28K from the BFCNs is a potential mechanism for the selective loss of these neurons in neurodegenerative diseases of the elderly, such as Alzheimer's disease.

T4.16
INCREASED GLUCOCORTICOID (GR) AND MINERALOCORTICOID (MR) RECEPTOR mRNA EXPRESSION IN HIPPOCAMPAL REGIONS OF ADLzheimer's DISEASE PATIENTS. I.A. Dubin, E.D. Hall, M.M. Boege, B.S. Purohit, A. H. Bouche. Dept. of Pharmacology, Univ. of Kentucky, Lexington, KY 40536-0084.
Corticosteroids have been implicated in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease. This study examined GR and MR mRNA expression in human post-mortem brain tissue from Alzheimer's disease (AD) patients and age-matched controls. Tissue was collected from 27 AD patients (ages 70-91 yrs; 12 men, 15 women) and 10 control patients (ages 75-32.5 yrs; 7 women, 3 men) from the ADRC at Sanders-Brown Center on Aging. Brodmann Area 9, hippocampus and entorhinal cortex tissue was collected and immunofixed in 4% paraformaldehyde. Tissue blocks were cryoprotected and sectioned (40 µm) at -17°C onto Vectabond coated slides. Stained and hybridized sections were counted (positive cells/100 µm²) using the MECD 34 imaging system. THA/THF-2 confirmed the presence of amyloid plaques and neurofibrillary tangles in AD brains. Following non-isotopic in situ hybridization of GR and MR mRNA, significantly higher levels of MR mRNA expression were observed in Area 9 from AD patients relative to controls (18.6±5.0 cells/1000 µm² versus 7.5±3.0 cells/1000 µm²). The CA1 region from AD patients exhibited a modest 44% elevation in MR mRNA expression compared to controls (32.7±2.51 versus 22.6±3.16 cells/1000 µm², respectively). Slightly elevated levels of GR mRNA expression was detected only in the CA1 region of AD brain (20.3±3.69 cells/1000 µm²) compared to GR mRNA message expression in the CA1 region from control tissue (16.5±1.45 cells/1000 µm²). This data suggests that altered expression of corticosteroid receptors could have a role in neurodegeneration observed with normal aging and AD. Progressive failure of calcium homeostasis initiated by glucocorticoid action is associated with cell loss in aging and AD. Our experiments indicate that individual variation in aging and AD is an important factor with regard to assessing mechanisms which underlie cell loss. (Supported by AG10836)

T4.17
17β-ESTRADIOL EXERTS NEUROPROTECTIVE EFFECTS ON SK-N-SH CELLS. M.N. Simpson, P.S. Green, G. Bishop and T. Nims,*, Center for the Neurobiology of Aging and the Dementias, Dept. of Molecular Pharmacology and Pharmacology, GRECC, VA Medical Center, University of Florida, Gainesville, FL.
Estradiol (E2) has been shown to exert organizational, neurotrophic and neuroprotective effects in the central nervous system. The present study assayed the specificity of the neuroprotective effects of estradiol for the potent 17β-isomer. SK-N-SH cells are a neuroblastoma cell line (APC, E) which we have always been estrogen-responsive, were cultured at low or high plating density. Cells were then exposed to 17β-E2 (0.2 or 1 mM), 17α-E2 (0.2 or 1 mM), cholesterol, testosterone, or dydrotestosterone (all at 2 µM) or progesterone androstene (0.2 to 200 nM). Cultures were treated with serum deprivation, which caused a profound loss of cell viability. Both 17α and 17β-E2 provided a dose-dependent protection of SK-N-SH cells at either plating density. By contrast, none of the other steroids tested protected cells from the insult of serum deprivation. The addition of progesterone (200 nM) to cultures depleted of estradiol repressed the expression of either isomer of estradiol at either dose. These results indicate that the neuroprotective effects of estradiol are not a general steroid effect and do not require the presence of estradiol isomers, as assessed by binding to cytosolic estrogen receptors or responses of peripheral estrogen-responsive tissues. As such, the neuroprotective effects of estrogen may be mediated by a non-genomic mechanism. (Supported by NIH AG10485 and Apollo Genetics, Inc.)

T4.18
Previously we have shown that a brief period of bilateral carotid occlusion (BCO) induced forebrain ischemia in gerbils triggers a progressive expression of amyloid precursor protein (APP), apolipoprotein E (APO-E), glial fibrillary acidic protein (GFAP), and β-amyloid (β-AP) in the selectively vulnerable CA1 region of the hippocampus. The increase in immunoreactivity is apparent to the degeneration observed in APP expression. Oxygen radicals and lipid peroxidation (LP) have been demonstrated to play a role in post-ischemic neuronal damage, and recent literature suggests a possible link between early oxidative stress and subsequent APP expression. Therefore, the present investigation examined the effect of a novel brain penetrating pyrrolopyrimidine LP inhibitor (U-10103E) on APP, APO-E, GFAP, and β-AP expression. Gerbils were treated at 30 mg/kg p. o. 30 min prior to the BCO and 2 hrs post-ischemia, followed by daily dosing for the next three days. U-10103E provided a significant decrease in the expression of all markers (p<0.05), which correlated with a significant effect on preservation of the CA1 neuronal cell population (p<0.05) as determined by cresyl violet histochemistry. Lipid peroxidation inhibitors may provide attenuation of various response proteins to ischemic injury, probably through reduction of neuronal cell damage.
1980
ALZHEIMER’S DISEASE: NEUROPHARMACOLOGY WEDNESDAY PM

T74.1
SUBSTANCE P POTENTIATES INTERLEUKIN-1- AND LPS-INDUCED RELEASE OF IL-6 FROM HUMAN ASTROCYTOMAS. G.E. Nolan, R.E. Jablon, R.B. Nelson. Department of Neuroscience, Pfizer Central Research, Groton, CT.

Astrocytes responding to optic nerve transection upregulate high-affinity substance P-binding sites (NK-1 receptors) within a month following the injury (PNAS 86:5193). The functional consequences of NK-1 receptor upregulation on reactive astrocytes are unknown. Because this receptor upregulation occurs in the context of an inflammatory response to brain injury, we sought to determine the involvement of neuroinflammatory factors in modulating reactive astrocyte function. We first determined (as previously reported) that in U373 cells SP alone causes a moderate dose-dependent increase in IL-6 release and intracellular calcium levels. Maximal effects are blocked by the non-Nepti-NK-1 antagonist CP-96345. The maximal SP-evoked IL-6 release is at least 10-fold less than the maximal release evoked with the pro-inflammatory agents IL-1β or lipopolysaccharide (LPS). However, we found that SP can interact synergistically with either IL-1β or LPS to potentiate increases in IL-6 release. These results indicate that substance P may potentiate cytokine-evoked release of IL-6 from reactive astrocytes. Since astrocyte overexpression of IL-6 in vivo has been shown to produce neurodegeneration, our findings may have therapeutic applications in neurodegenerative diseases involving chronic CNS inflammation, such as Alzheimer’s disease, multiple sclerosis, and Parkinson’s disease.

T75.1
TROPHIC SUPPLMENTS OF MESENCEPHALIC CELLS IN HIBERNATION MEDIA ENHANCES SURVIVAL OF DOPAMINE NEURONS. F.M. Caruso*, L.S. Pak and L.B. Nelson. Research Center for Brain Repair, Rosal-Presbyterian-St. Lukes M.C., Chicago, IL 60612.

Mesencephalic neurons destined for transplantation into the striatum of patients with Parkinson’s disease (PD) are generally held in hibernation at 4°C as a means to reduce metabolism and improve survival rates. In the current study, various substances were added to the hibernation media at 4°C to determine if any would improve survival rates of PD neurons. For example, we added PAC-sensitive NGF (PAC) or BA to the hibernation media for various time periods. All supplements improved survival rates. The most effective supplement was PAC-sensitive NGF. These results indicate that PAC-sensitive NGF may improve survival rates of PD neurons in hibernation media. Thus, PAC-sensitive NGF may improve survival rates of PD neurons in hibernation media.

T75.3
STRIAL TRANSLANTATION OF MICROENCAPSULATED BOVINE CHROMAFFIN CELLS REDUCES ROTATIONAL BEHAVIOUR IN THE RAT MODEL OF PARKINSONISM. P.W. Tang, H.C. Kan* and A.M. Sub Department of Physiology, University of Toronto, Toronto, ON, AIDS IAS CANADA.

Strial transplantation of dopamine-producing tissue has been proposed as a possible treatment for Parkinson’s disease. To avoid the ethical issues associated with the use of human fetal tissue, transplantation of chromaffin cells from the adrenal gland has been suggested; however, survivability of these transplants is low. In our study, we assess the efficacy of bovine chromaffin cell transplants immunosuppressed within a perisclerotic biocompatible polymer membrane vs. free cell transplants in the rat model of parkinsonism.

In vitro studies compared levels of high-potassium depolarization-evoked release of catecholamines from free chromaffin cells vs. microencapsulated within alginate/poly-L-lysine microcapsules (APA) capacity. Results of a perfusion study showed comparable levels of release from both free and encapsulated cells: 0.6 × 10^8 encapsulated cells released approximately 4700 ng of norepinephrine, 5000 ng of adrenaline and 350 ng of dopamine. In vivo studies showed that the use of APA was beneficial in reducing the amount of catecholamines released. Notably, the use of APA significantly reduced the amount of catecholamines released in the striatum and substantia nigra. These results suggest that APA can be used to improve the survivability of chromaffin cell transplants in the rat model of parkinsonism.

T75.4
CHARACTERIZATION OF HSV-1-EXPRESSED TYROSINE HYDROXYLASE IN A NON-DOPAMINERGIC CELL LINE. F. Serrano*, M.A. Benedict, W.F. Goins, G.M. Zigmund, J.A. Gliozzi* and T.G. Adams. Department of Neuroscience and 1Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15260.

The delivery of foreign genes into CNS neurons may aid in the treatment of certain neurodegenerative diseases, such as Parkinson’s disease. Herpes simplex virus (HSV-1) is one tool that demonstrates promise for this function. We have previously presented data on our design and construction of a non-replicating HSV-1 vector, and the results showed that this vector can increase the production of tyrosine hydroxylase (TH) in non-dopaminergic cells in culture. In order to determine the specificity of this transduction, we used a variety of non-dopaminergic cells as targets. We found that the level of TH expression in these cells is proportional to the level of viral gene expression. Thus, this vector can be used to increase the production of TH in a variety of cell types.

This work was supported by the Medical Research Council of Canada.

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Defective herpes simplex type 1 (HSV-1) viral vectors have been widely used for in vivo gene transfer in the central nervous system (CNS). Whereas the duration of gene expression directed by the viral promoters in ampiclon vectors is short-lived, we constructed an ampiclon vector in which gene expression was directed by the tyrosine hydroxylase (TH) promoter. When the upstream DNA sequence of the rat TH gene, previously shown to direct tissue-specific expression in transgenic mice, was fused to the HSV ampiclon (THlac), the viral IE4/5 promoter (HSVlac) was used as a control. Sprague-Dawley rats received unilateral subcutaneous injections of virus stock into substantia nigra (SN). To determine retrogradely transported expression, virus stock was injected stereotaxically into striatum. After three weeks rats were sacrificed for N-gal histochemical and TH immunocytochemical analysis. In rats receiving viral injection of THlac, expression of the LacZ gene was observed in neurons as well as glia. In rats receiving striatal injections, blue cells were also observed in SN pallidal to injection side. These blue cells were nigral dopaminergic neurons evidenced by double staining with TH immunocytochemistry. In contrast, neither nigral nor striatal injection of HSVlac resulted in positive results. This study demonstrates that TH-directed neuronal expression, but not a viral promoter, will direct longer-term expression in adult brain. Supported by NIH grant, MH22485.


Parkinson's Disease is a debilitating illness characterized by degeneration of the dopaminergic neurons in the nigrostriatal system. The greater omentum possesses an abundant supply of dopamine and is capable of developing cortical vascular connections when transplanted to the brain. We investigated the potential benefits of transplanting a pedicled graft of the greater omentum to the brain as a potential therapy in a Parkinson's animal model (PAM). Since the omentum might increase the supply of dopamine to the corpus striatum and retard progression of the motor deficit. Sprague-Dawley male rats (60 days of age) were divided into four groups: 1) a control group, 2) a sham group, 3) a PAM Oil group, and 4) a PAM omental group. PAMs were produced by recombiant adenoviral administration of f.f-hydroxydopamine (6-OHDA). Amorphophine induced rotational studies were conducted weekly to monitor the progression of The motor deficit. Following two weeks of behavioral testing, the PAM Oil group received amphetamine followed by cyclosporin over the next 5 weeks to suppress the motor deficit. After surgical procedure, the proximal end of the pedicle was brought under the subcutaneous tissue and transposed to the surface of the cortical area at the level of the parietal-temporal lobe jucnture. Amorphophine induced rotational studies were continued an additional three weeks. The results shows that the number of rotations was significantly less (<0.001) in the PAM omental group when compared to the PAM Oil group. The results suggest that pedicled omental graft might be effective in ameliorating the progression of a PAM.


We have studied the effect of the lesion of the globus pallidus medialis (GPM) in 3 monkeys rendered parkinsonian by intravenous administration of MPTP. The monkeys were allowed to recover from the last MPTP injection up to 6 months and they did not receive any dopaminergic agents. Fine motor tasks were performed in all animals before MPTP administration and immediately before surgery, when the degree of parkinsonism was considered stable.

Unilateral posteroventral palidotomy was performed in 2 monkeys while in the other animal the lesion was made in both GPM. The lesion of the GPM was made by injecting 1-2 pl of 50 mm kainic acid using a modified David Koff stereotaxic frame. Unilateral lesion of the GPM induced an contrastralateral amelioration of the motor deficits of fine motor tasks, but mild contrateral otal achor was also observed, which persisted up to 3 months. Bilateral lesion of GPM induced severe generalized dyskinesia with improvement of the parkinsonian motor deficit. Post-mortem analysis showed the lesion was confined to the posterior part of the GPM, but a cell depletion was also observed in the dorsolateral part of the subthalamic nucleus homolateral to the lesion.

CHARACTERIZATION OF FIBROBLAST CELLS GENETICALLY MODIFIED TO PRODUCE L-DOPA IN VITRO AND IN VIVO. C. Benečak*, S. R. Michel, D. Young and U. J. Kang. Dept. of Neurology, University of Chicago, Chicago, IL 60637.

In previous studies, we have generated cells (FFK1THGC) which produce L-DOPA by doubly transducting primary fibroblasts with the cDNAs for tyrosine hydroxylase (TH) and GTP cyclohydrolase I (GTPCHI). These cells produce L-DOPA spontaneously since they produce tetrahydrobiopterin (BH4) which is the cofactor for TH.

To examine dopamine synthesis in vitro, FFK1THGC cells were cocultured with fibroblasts engineered to produce aromatic L-amino acid decarboxylase (FFK1AADC). Cocultured cells were grown with media containing 0.01% of ascorbic acid for one hour. This media was analyzed for L-DOPA and dopamine (DA) by HPLC-ED. FFK1AADC and FFK1THGC were cocultured with FFK1AADC cells, there was a reduction in L-DOPA and dopamine which was detected in the media.

These FFK1THGC cells were grafted into the striatum of rats depleted of DA by 6-OHDA. Immunostaining for TH in FFK1THGC cells was more robust compared to FFK1TH grafts, suggesting that BH4 may have another role in addition to acting as a necessary cofactor for its enzymatic activity. Further in vivo studies examining biochemical and behavioral effects of these cells are in progress.


In a previous publication (Baev K.V., Neurol. Res. 1994, v.17, 38-48) it was suggested that the pharmacological basal ganglia-thalamocortical loop's can be considered as a functional system that models the behavior of the body and the environment during movement control. This predictive mechanism has to be turned on the controlled object in order to model precisely, i.e., it has to be learning system. The parameters of this system are tuned on the object behavior using an error signal that comes from the substantia nigra pars compacta. Minimization of the error signal is the final stage of learning process.

Therefore, Parkinson's disease (PD) has to be considered to result from degeneration of the error distribution system. Based on this theory, we make the conclusion that functional neurosurgical procedures such as pallidotomy or thalamotomy trick the control system in such a way that it does not recognize an error in its prediction anymore. Lesions placed in the pallidum or its projections to the thalamus make predictive signals less important so that real error flow from the controlled object prevails. After placement of a lesion in nonplastic thalamic projections, the system primarily chooses predictive model afferent flow to determine the current state of the object. Therefore, these surgical procedures have to be considered symptomatic static treatments. There is no real improvement of system function, only alleviation of the symptoms of PD. Chronic stimulation of basal ganglia circuitry through implanted electrodes has to be considered as adding noise to the system in addition to a functional block that it places at the stimulation site. Noise helps the system to slide down along an error surface to its global minimum when the model correctly decodes the object behavior. Other methods of treatment of PD such as transplantation will be also discussed.

THE MECHANISM OF ACTION OF PALIDOTOMY IN PARKINSON'S DISEASE (PD): CLINICAL AND PHARMACOLOGICAL RESPONSE. G. Lasagna, J.A. Deora, A. Geoghe, E. Rami, R. Bakay, M. DeLong, J. Vink*, Clinica Quito, San Sebastian (Spain) and Emory University Hospital, Atlanta, GA 30322.

Six patients with PD were evaluated before and 3-6 months after microelectrode guided unilateral pallidotomy. Rigidity and akinesia were measured individually for each body segment, and arm and leg timed tapping tests were scored at baseline and after 24-72 hours without drug. The pharmacological tests assessed the duration of the motor response (ON) to Sinemet (250/25 mg) and suscousum apomorphine (2.5 mg) and the severity and type of dyskinesia. Pallidotomy induced a marked reduction in akinesia and rigidity mainly contralateral to the lesion. The duration of the response to Sinemet was significantly prolonged and dyskinesia disappeared completely. Some body segments, i.e., shoulder, showed a permanent improvement after 24-72 hours without medication. Pallidotomy shifts the pharmacological response toward characteristics associated with milder severity of parkinsonism. The response to dopaminergic drugs is blocked by pallidotomy.
DEGENERATIVE DISEASE: PARKINSON'S—TRANSPLANTATION, PALLIATORY AND IMAGING WEDNESDAY PM

T75.11
THE MECHANISM OF ACTION OF PALLIATORY DRUGS IN PARKINSON'S DISEASE (PD): PHYSIOLOGICAL AND IMAGING STUDIES. L.A. Quinn*, J.L. Roberts-Loeber, A. Ceballos-Baum, N. Leenders, D. Brooks, P. Arestel, G. Linazasoro, J. Ourch, B. Bakay, J. Vink, M. DeLong, Clino Quirin, San Sebastian, Spain; MRC Motor and PET Units, London (UK), Paul Scherrer Institute, Villigen (Switzerland), Emory University Hospital, Atlanta, GA

Six patients with PD were evaluated before and 3-6 months after microencapsulated gold unilateral pallidotomy. Laboratory assessment included recording EEG premenopausal, magnetic cortical stimulation of the motor cortex, intracarotid L-[14C]-tyrosine infusion taken while the subject performed sequential hand movements. Following pallidotomy, all patients improved clinically but hand function remained unchanged in two, PET studies showed a significantly increased activity of the supplementary motor area (SMA), areas 6 and 4 and subthalamic pretoral cortex in 4 patients. The IOI for sequential hand movements was reduced by 44% and simple RT was decreased by 34%. There were no significant changes in the LSLK, blink reflex and motor cortex recovery curves and choice RT. Restoration of premotor and dorsolateral pretoral cortex activity appears to be an important mechanism mediating the effect of pallidotomy in PD.

T75.12

The graphical analysis of PET data permits to estimate the striatal uptake rate constant (K) of an extended model which made it possible to estimate the rate of loss of striatal signal (Kloss) during FD PET studies. Kloss is an index of the release, metabolism and outward diffusion of fluorodopamine and its metabolites. We examined the rate of loss of striatal signal in FD PET studies in normal and MPTP-treated rhesus monkeys to explore the hypothesis that dopamine (DA) metabolism is altered.

Four unilaterally MPTP-treated rhesus monkeys and 4 age-matched controls were scanned in an Ecat 9323/31 tomograph for up to 4 hrs after injection of 5 mCi of FD. Metabolite analysis was performed using an alanin-internal standard with anion/cation exchange columns. The extended graphical analysis was used to calculate Kloss. Kloss was significantly decreased (p < 0.05) higher in the MPTP-treated striatum (0.00573 ± 0.002 min^-1) compared to the unlesioned striatum (0.00243 ± 0.00069 min^-1) and controls (0.00258 ± 0.00069 min^-1).

These results are in keeping with the hypothesis of compensatory increase in DA turnover in parkinsonism. Kloss as measured with FD PET may be used to explore DA presynaptic compensatory mechanisms.

T75.13
PET [18F]-fluoro-L-m-tyrosine Imaging of MPTP-Induced Parkinsonism. W.J. Jagust*, J.L. Ebeling, S. Jordan, H.F. VanDyck*, J.P. O'Brien, M. Emborg, D. Rumsey, M.S. Bankiewicz, Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720; Somatix Therapy Corp., Alameda, CA 94501

PET studies using the dopamine tracer, [18F]-fluoro-L-dopa (FD) have been extensively used to study Parkinson's disease. Interpretation of these studies has been limited because of the potential contributions of labeled metabolites that readily enter the brain. Here we report the results of PET studies using the tracer [18F]-fluoro-L-m-tyrosine (FM) in monkeys before and at weekly intervals after unilateral intracarotid administration (ICA) and i.v. MPTP administration. Animals were injected with approximately 10 mCi of FM and were imaged for approximately 2 hours. Prior to PET administration, all animals showed preferential accumulation of the tracer bilaterally in the striatum and midbrain, but little accumulation in other brain regions. Following unilateral ICA MPTP administration (3 mg MPTP- HCl), we observed decreased signal in the striatum on the lesioned side and increased signal in the nigra/VTA complex. After i.v. MPTP administration (0.5 mg/kg) we observed a further reduction in signal in the striatum on the previously lesioned side, as well as a reduction on the contralateral side. Data were analyzed using a 3 compartment model with 5 rate constants to describe the time course of radioactivity in the tracer in the striatum. Prior to MPTP administration the mean value for k5, describing FM deactivation, was 0.06 (sd = 0.01) for the patiens. Following unilateral ICA MPTP administration, k5 for the patiens was 0.03 (sd = 0.01) for the lesioned side, and 0.04 (sd = 0.01) for the contralateral side. After i.v. MPTP administration there was a further decrease in k5 for the previously lesioned patiens (mean k5 = 0.3, sd = 0.0001), as well as a decrease on the contralateral side (mean k5 = 0.02, sd = 0.01). These values corresponded to the extent of dopaminergic denervation. FM may be useful as a tracer for evaluating dopaminergic function which are effective for tracking MPTP lesions and should be helpful in detecting grafts of genetically modified cells in this model.

T75.14
CHARACTERISTICS OF 18 F-labelled ligands for Dopamine and Dopamine Transport Function Using Positron Emission Tomography (PET). Bakay RAE*, Goodman MM, Boyer KL, Watts RW, Byrd L, Hoffman JM, Departments of Neurology and Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322.

In order to develop and characterize Dopamine and Dopamine transport function in CNS transplantation by non-invasive techniques, a series of nonhuman primate studies were performed using PET. Fluorodopa is a standard for evaluating the dopaminergic systems, but has a high (O2:C) to-2 ratio. Dopamine transporter complex is important in the regulation and synthesis of Dopamine, and is diminished in Parkinson's disease. Two distinct isotopes have been developed: fluoro-2-propyl-chloro-ethyltyramine (FPCT) and fluoro-isopropyl-chloro-tyramine (FIPF). These compounds were synthesized to minimize nonspecific binding and achieve maximum specific activity. Nonhuman primate studies have been performed that characterize both blood and brain kinetic behavior of these isotopes. In combination with fluorodopa, it will be possible to completely characterize the uptake, binding, incorporation, release, and reuptake of Dopamine in vivo using PET. Data from normal, MPTP-lesioned, surgical controls and fetal mesencephalic grafted nonhuman primates will be presented.

Supported by NS-24340 and R-001049.

T75.15
ELEVATION OF TYROSINE HYDROXYLASE (TH) mRNA EXPRESSION AND FLUORO-META TYRASONE (FM) SIGNAL MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET) IN THE SUBSTANTIA NIGRA (SN) AFTER MPTP ADMINISTRATION IN MONKEYS. D. Nagy*, J.L. Ebeling, W. Jagust, S. Jordan, M.S. Bankiewicz, W.W. McAuliff and M.S. Bankiewicz, Somatix Therapy Corporation, Alameda, CA 94501, Lawrence Berkeley Laboratory, UC Berkeley, CA 94720.

Administration of 0.4 mg/kg of MPTP into one internal carotid artery (ICA) produces hemiparkinsonism (HDP) in monkeys (m). In this study we examined TH mRNA expression in normal and HDP m, while 3 other m. underwent PET scanning after MPTP administration and at weekly intervals after ICA and i.v. MPTP administration. The m received an intracarotid injection of 3 mg of MPTP- HCl supplemented with 0.3 mg/kg i.v. to induce general parkinsonian signs in addition to HDP. These animals were able to sustain themselves without any L- Dopa administration. The in-situ hybridization (ISH) techniques were applied for the detection of the TH mRNA content of the midbrain DA cells using 125I labelled complementary RNA probes. Adjacent sections were stained for H&E and examined for TH-immunoreactivity (TH-IR). In the midbrain, the side ipsilateral to MPTP infusion very few TH-IR cells were present and contralateral SN appeared to be partially lesioned. TH mRNA expression was found higher in the SN on the previously lesioned side of the midbrain. An increase in the TH-IR was detected in the SN of the contralateral side of the m examined with PET there was an increase of signal in SN at 7 days after ICA MPTP on treated side and at 7 days on the contralateral side after i.v. MPTP injection. High levels of the TH-mRNA expression of the MPTP treated animals and increase of FMT signal at 7 days after MPTP administration suggests up-regulation of the remaining DA cells. These findings are consistent with increased IV-DAA transporter expression and a compensatory mechanism of the remaining and/or degenerating DA cells in SN.
### T76.1

**ALTERATIONS OF LONG-TERM POTENTIATION (LTP) AND PAIR-PULSE POTENTIATION (PPP IN VIVO RAT HIPPOCAMPAL DENTATE FOLLOWING DEVELOPMENTAL LEAD EXPOSURE)**

**D.T. Ruan**, C. Zhao, G.B. Beol; X.Y. Tang, J. Chen, K. Zhao and Y.Z. Xu

Dept. of Biology, Univ. Sci. Tech. China, P.R.China

Objective: To examine the effects of lead exposure on hippocampal LTP and PPP using the novel sniffing assay.

Methods: Subjects included 40 rats, with 20 subjected to lead exposure and 20 serving as controls. Lead exposure involved exposure to lead acetate in drinking water for 21 days postnatal. The sniffing assay was performed using the novel sniffing apparatus.

Results: Lead exposure resulted in a significant reduction in both LTP and PPP compared to controls. The decrease in PPP was more pronounced than that observed for LTP.

Conclusion: Lead exposure during development affects hippocampal plasticity, as evidenced by reductions in LTP and PPP.

### T76.2

**ROLE OF APOPTOSIS IN DELAYED NEURONAL DEATH FOLLOWING A BRIEF EXPOSURE TO PRIMARY CULTURES OF CEREBELLAR GRANULE NEURONS TO ZINC**:


ASRI, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Philadelphia, PA 19121.

In primary cultures of rat cerebellar granule neurons, a brief, 15 min exposure to zinc (300 μM and higher) resulted in delayed neuronal death. Cell death was assayed by measuring mitochondrial viability with the MTT assay and cell membrane integrity with the trypan blue exclusion assay. These experiments suggest that apoptosis may play a role in the delayed death of these neurons.

### T76.3

**NEUROTRANSMITTER RELEASE IN PC 12 CELLS EXPOSED TO LEAD**


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In children, exposure to low levels of lead (Pb) results in learning deficiencies. One hypothesis to explain this observation is that Pb impairs learning by disrupting mechanisms that control the release of neurotransmitters.

The relationship between Pb and neurotransmitter release was examined by studying norepinephrine (NOR) release in PC 12 cells by using a method that takes advantage of the reuptake of [3H]noradrenaline into vesicles. A 60 min incubation with 20 μM (CH₃COO)₂Pb, but not the chloride salts of Cd, Zn or Mn resulted in the release of NOR. The minimum concentration of Pb needed to provoke NOR release was 20 μM. However, if the cells were stimulated with 100 μM of noradrenaline (CA-ATPase) or with activators of protein kinase C (PKC) the minimum concentration of Pb was reduced to 10 μM and 5 μM, respectively.

Neurotransmitter release was also accompanied by the release of two proteins found in the extracellular, secretogranins II and chromogranin B. A 200 μM concentration of Pb or Ni effectively blocked the release of NOR in PC 12 cells stimulated with an inhibitor of Ca-ATPase but was relatively ineffective in cells stimulated with a PKC activator.

We conclude that PC 12 cells exposed to Pb release neurotransmitter. Furthermore, there appears to be at least two pathways that will heighten a cell's sensitivity to Pb, one that involves microsomal Ca-ATPase and another that uses PKC.

### T76.4

**LOW LEVEL LEAD EXPOSURE DURING DEVELOPMENT: DOSE-DEPENDENT NEUROBEHAVIORAL CHANGES IN THE RAT**


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We investigated the effects of incremental doses of low-level Pb acetate or sodium acetate exposure during development on runway maze performance in Binghamton heterogenous Stock (HE) mice. Pb acetate was either untreated or received at 0, 5, or 10 mg/kg Pb acetate via intragastric intubation on postnatal days 6, 9, 12, 15 & 18.

No overt effects of Pb on physical development, including growth, were detected in any groups. When tested at 40 days of age, Pb-exposed mice exhibited a dose-dependent increase in the proportion of runway errors - with effects emerging at levels as low as 10 mg/kg. These data support a growing evidence that very low levels of Pb exposure during development may impair cognitive performance later in life.

**NEUROTOXICITY: HEAVY METALS**
NEUROTOXICITY

776.5

The prominece of neuromelanin in humans, and its accumulation extracellularly in Parkinson disease and other extrapyramidal disorders involving the substantia nigra, has led to speculation that neuromelanin might itself be neurotoxic. Iron is known to accumulate in the substantia nigra of Parkinson's patients, and it has been shown that neuromelanin binds iron. Therefore, it has been shown even more specifically that iron in neurotoxic when complexed with melanin. The proposed mechanism of neurotoxicity involves iron, leading to neuronal death from toxic oxygen species. In order to test this hypothesis, as well as to learn the pathways capable of inducing the cytotoxicity of melanin in humans, we have attempted experiments using human substantia nigra.

Although some TH-positive neurons were destroyed by trauma in the needle tract area in both control and melanin injected rats, TH-negative neurons appeared to be completely unaffected by melanin at all time points. There was no difference in results between normal and Parkinsonian melanin. Some melanin could be seen within a few 4.5 pm positively melanin at all time periods, but most melanin seemed to be ignore the melanin granules. The present reports show that human neuromelanin is not toxic to rat substantia nigra or caudate putamen neurons and it remains for long time periods in brain.

776.7

Pb2+ is a potent inhibitor of NMDA receptors and its action is dependent on the age of the neuron. We tested the hypothesis that the vulnerability of NMDA receptors to Pb2+ blockade is dependent on the receptor subunit composition which changes during development. Concentration-response curves for NMDA and glutamate were generated in the presence and absence of Pb2+ using two electrode voltage-clamp technique and NMDA receptors consisting of 1G1, 2G1 and 2G2 subunit combinations expressed in Xenopus laevis oocytes. For all three subunit compositions glutamate was a more potent agonist than NMDA. In oocytes expressing 1G1, 2G1 and 4G2 subunits Pb2+ inhibited glutamate-activated currents with IC50s of 0.87 ± 0.25 mM; 1.21 ± 0.22 mM; 6.1 ± 0.55 mM and NMDA-activated currents with IC50s of 1.37 ± 0.47 mM; 1.11 ± 0.33 mM; 3.04 ± 1.54 mM (mean ± S.E.). Pb2+ reduced the maximal current amplitude but did not significantly alter the IC50s for glutamate or NMDA consistent with a noncompetitive block. The IC50s for Pb2+ blockade of NMDA- or glutamate-activated currents were significantly larger for 1G12G2 subunits when compared to 1G1 or 2G2 combinations. These data suggest that brain regions with 1G1 or 1G2 NMDA receptors subunits would be more vulnerable to Pb2+ toxicity than those with 1G12G2 NMDA-receptors. (Supported by NS19611)

776.9
BRAIN LIPID PEROXIDATION AND POLYDIPSIA DURING IRON OVERLOAD IN RAT. Carol A. Gunnet, James L. Hargrove, and Diane K. Hatte*. Dept of Pharmacology/Toxicology and Foods and Nutrition, The University of Georgia, Athens, GA 30602.

Brain membranes are composed of a high percentage of polyunsaturated fatty acids relative to other membranes. The fatty acids themselves are therefore highly peroxidizable and at risk for oxidative free radical damage if local oxidative stress overcomes the brain's relatively moderate level of antioxidant defences. The role of iron in the blood brain barrier is potential to protect the brain continually from oxidative stressors in the blood. Free iron catalyzes lipid peroxidation via Fenton reactions in vivo. Iron-catalyzed free radical formation initiates lipid peroxidation. The purpose of this study was to produce a long term oxidative stress on brain and peripheral organs using ferrous chloride (20 mg iron/kg, ip.) and determine the extent of 1 lipid peroxidation and 2 glutathione levels in brain, liver, kidney and heart.

Summary of results: Iron overload treatment caused rapid and profound polydipsia. The polydipsia was initiated within minutes of injection. Iron overload produced significant brain, liver, kidney and heart lipid peroxidation. Early time points had higher levels of lipid peroxidation relative to the 2 hr time period, presumably following rapid sequestration of free iron. Glutathione levels were depleted in brain. Gamma-Glutathione S-transferase (GSH) was reduced in JH, APPE Fellowship to CAG and Ga AHA.

776.6
MANGANESE DECREASES GLUTamate UPTake IN CULTURED ASTROCYTES. A.S. Hase1 and M.D. Norenberg. Departments of Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine and VA Medical Center, Miami, FL 33101.

Manganese has recently been shown to accumulate in the basal ganglia in patients with chronic hepatic encephalopathy (HE). Ammonia and astrocytes have been strongly implicated in the pathogenesis of HE, and since we have recently shown that manganese decreases glutamate uptake in cultured astrocytes, we examined whether manganese also affected glutamate uptake. Ccultured astrocytes from newborn rats were treated with manganese (II) chloride (1.5-50 mM) in the absence or presence of ammonium chloride (5 mM), and glutamate uptake was determined using the non-metabolized glutamate analogue [3H]D-aspartate. Exposure times varied between 1 and 2 days. Treatment with manganese (50 mM) for 2 days resulted in a 11% decrease in transport of radiolabel into cells compared with controls (control 11.13 ± 2.37 vs. Mn+ 9.91 ± 2.66), while treatment with ammonia alone resulted in an 18% decreased uptake of [3H]D-aspartate after 48 hrs. In the presence of both manganese and ammonia, a further lowering of D-aspartate uptake was observed (34%). Preliminary data also suggested greater sensitivity of striatal cultures to the effects of manganese on glutamate transport. These results suggest that manganese alone has a detrimental action on glutamate transport into astrocytes which is additive to the ammonia effect, and may contribute to the pathogenesis of hepatic encephalopathy. (Supported by NIH grants DK31853, NS02921, VA and GRECC)

776.8
ALTERATIONS IN N-METHYL-D-ASPARTATE (NMDA) RECEPTOR SUBUNIT EXPRESSION AFTER LEAD TREATMENT IN NEONATAL RATS. M.A. Wilson*, J. Brasier, M.V. Johnston and G.W. Golden, Neurosciences Laboratory, Kennedy Krieger Research Institute and Dept. of Neurology, Johns Hopkins University, Baltimore, MD 21205.

One of the primary sites of action of the environmental neurotoxin lead is at the NMDA receptor (Allanond et al., '90), lead subacutely inhibits NMDA currents, acting as a non-competitive antagonist of both NMDA and glycine (Gularte & Mccoli, '92, Uenew et al., '93). We have examined the effects of lead exposure lead on NMDA receptor subunit expression in rat pups. Rat pups were administered 0.2% lead acetate in their drinking water, beginning on the day when their litters were delivered. Lead-treated and untreated control litters were cullled to 8 pups at birth. Rat pups were decapitated on postnatal day 15, and in situ hybridization with oligonucleotide probes for NR1, NR2A and NR2B was used to evaluate NMDA receptor subunit expression in the hippocampus. Expression of NR2A mRNA was significantly reduced in the granule cell layer of the dentate hilus (p<0.01) and in the pyramidal layer of the CA1 region of the hippocampus (p<0.05). No significant changes in NR1 or NR2B expression were observed. The selective effect of lead on dentate granule cell is of special interest because these neurons have a mechanism for accumulation of the putative co-transmitter zinc that may serve to concentrate lead in this part of the hippocampus. Because NR2A expression normally increases in the forebrain postnatal day 7 and 15, the reduced expression observed in lead treated rats may represent a developmental delay in maturation of excitatory amino acid receptors. (Support: The Robert Loet and Clara Guthrie Patterson Trust, NIEHS Grant 02380)

776.10
EFFECTS OF IN VITRO CHRONIC LEAD EXPOSURE ON CA2 CURRENTS IN BASAL FOREBRAIN NEURONS FROM ADULT RATS. C.A. Grover*, M.J. Juske, W.H. Griffith, & D.L. Price, Dept. of Medical Pharmacology & Toxicology, HSC, Texas A&M University, College Station, TX 77843-1114.

It is well known that in vitro acute lead treatment inhibits neuronal calcium currents. We studied the effects of chronic in vivo lead exposure on LVA and HVA calcium currents in in vitro basal forebrain neurons. Acute male Sprague-Dawley rats were given 500 ppm lead acetate (Group Lead) or pair-fed sodium acetate (Group Control) in the drinking water for 65-75 days prior to in vitro electrophysiological experiments. Whole-cell patch-clamp techniques were performed on acutely isolated neurons from the basal forebrain. Current-densities were determined for all cells by normalizing Ca2+ currents (pA) for capacitance (pF), and current-voltage relationships were determined. From a holding potential of -40 mV Ca2+ currents were generated using a 2 sec prepulse to either -100 or -50 mV followed by voltage steps between -90 to +30 mV. There was no significant difference in maximum peak current-density between cells from Control (X = 6.6 ± 5.11 pA/pF, n=50) and Lead (X = 5.5 ± 5.15 pA/pF, n=27) treated animals. In a subset of cells, rundown of maximum steady-state current-densities was significantly greater for Control (X = 56.6 ± 6.6 %, n=10) than Lead rats (X = 22.8 ± 10.26 %, n=10). These findings may suggest that chronic exposure to lead, like acute intracerebral injection of lead acetate (Sorg et al, Neurosci. Abstr., 20:1719; 1994), causes a reduced rate of calcium current rundown. Supported by ES05639 (CAG), AA06322 (GDP) and AG07805 (WHG).
T76.11 METHYLmercury (MeHg)-INHIBITED INDUCTION OF WHOLE CELL POTASSIUM (K⁺) CURRENT IN RAT CEREBELLAR GRANULE CELLS. J. Singal, and W.D. Atchison. Dept. of Pharmacol, Toxicol, Inst. of Environ, Toxicology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

MeHg is a neurotoxic metal which interacts with Na⁺ and Ca²⁺ channels in a potent and apparently irreversible manner. Cerebellar granule cells are particularly sensitive to the in vivo effects of MeHg and provide an excellent model for studying MeHg effects on ion channels in vitro. The whole-cell patch clamp technique was used to examine peak and steady-state K⁺ currents following exposure of cerebellar granule cells to 0.23 μM MeHg. The extent of block was similar following either strong (+120 mV) or relatively weak (-10 mV) depolarizations, demonstrating that the effect is voltage-dependent. Initial experiments designed to examine the effect of MeHg on K⁺ current activation and inactivation showed that MeHg did not alter the voltage at which the current activates, nor did it affect the rate at which the current inactivated. This observation does not appear to be specific to MeHg, as similar effects are seen with other drugs known to be sensitive target of MeHg following in vitro exposure. Supported by NIH grant ES03299.

T76.13 MICROMOLECULAR CONCENTRATIONS OF METHYLmercury (MeHg) BLOCK VOLTAGE-ACTIVATED CALCIUM-, SODIUM- AND POTASSIUM CHANNELS OF THE RAT HIPPOCAMPUS IN VITRO. R. Leonard, P. Hahn and D. Bässig. Physiology II, H-Heins University, 40225 Düsseldorf, Germany.

Methylmercury (MeHg) readily accumulates in the nervous system and is known to cause a wide variety of neurologic effects, ranging from aberrant reflexes to loss of coordination and learning deficits. The whole-cell patch clamp technique, we examined voltage-activated ion currents of rat dorsal root ganglion (DRG) neurons. Cells were obtained from 3-day old rat pups and cultured for up to 5 days. Voltage-activated calcium-, sodium- and potassium channel currents were separated by selective blocking agents and specific deproteinizing voltage steps. Calcium channel currents were carried by barium (10 mM). Subtypes of the ion currents have not been distinguished. MeHg had no effect on either of these currents. The extracellular solution just before the beginning of each experiment. All currents were leak corrected by a p4 protocol. Dose-response relationships were calculated by fitting the data to the Hill equation. All three types of voltage-activated ion currents were reduced by MeHg in a concentration-dependent manner. Voltage-activated calcium (IC₅₀ = 6.5 μM) and potassium channel currents (IC₅₀ = 2.2 μM) were shown to be more sensitive to MeHg than voltage-activated sodium currents (IC₅₀ = 12.1 μM). The calculated Hill-coefficients were -1 for the blocking of calcium currents and -1.7 for the blocking of sodium channels. The reduction of calcium and sodium channel currents appeared to be dependent. Indepedent of the external solution used, in some cases the application of higher concentrations of MeHg (>5 μM) resulted in a biphasic change of the resting membrane current. None of the above described effects was reversible. These results indicate that the blocking of voltage-activated ion currents may contribute to the neurotoxicity of MeHg.

T76.16 STUDIES ON THE MECHANISM OF METHYLmercury (MeHg) TRANSPORT INTO THE BRAIN. R. Park, S. Yee and B. H. Choi. Neurpathology Unit, University of California, Los Angeles.

A previous study in our laboratory as well as those of others have demonstrated that transport of MeHg into brains of mature rats is enhanced by co-administration of 3-aminobenzamide. However, no significant enhancement of brain Hg uptake was noted following injection of 0.1 M methylmercury 20 minutes prior to 0.05 mM MMC injection in C57BL/6 mice. Whereas injection of MMC-conjugated MMC conjugates, significantly enhanced brain cyclic AMP, separate injections of 20 mg/kg body weight of MMC and 30 μl of 75°C-cytochrome at 20 min. interval showed no significant enhancement of brain cyclic AMP as compared to controls. To further examine the mechanism of MeHg transport into the brain, varying doses (1, 2, 4, 6 and 8 μM) of methylmercuric chloride (MMC), MMC-cysteine, MMC-glutathione (GSH) and MMC-beta-mercaptoethanol (BME) were injected intracerebrally into C57BL/6 mice, and brain Hg uptake determined 5 hours thereafter. Dose-dependent increase of Hg uptake was noted in all groups. However, significantly enhancement of brain uptake was found in groups injected with MMC-conjugates (MMC-BE=MMMCysteine=MMC-BE-GSH=MMMC). The kinetics of brain Hg uptake between MMC and MMC-BE groups also differed considerably. These data indicate that the size and polarity of MMC-conjugates greatly influence MMC transport into the brain, and that MeHg transport into mature brains appears to take place through diverse and complex uptake systems. (Supported in part by NIHES grant ES 02989)

T76.12 PATHWAYS MEDIATING Ca²⁺ ENTRY INDUCED BY METHYLmercury IN CEREBELLAR GRANULE CELLS. M. S. Manty and W. D. Atchison. Dept. of Pharm. & Toxicol, Michigan State University, East Lansing, MI 48824.

Methyl mercury (MeHg) is a known neurotoxin, hypothesized to cause cerebellar granule cell degeneration by altering Ca²⁺ homeostasis; however, the mechanism of this effect is poorly understood. Prior work demonstrated a MeHg-induced biphasic rise in intracellular Ca²⁺ ([Ca²⁺]) with a large component of this rise due to influx of extracellular Ca²⁺. We have now measured the time course of the extracellular concentration of Ca²⁺ ([Ca²⁺]_{ext}) following exposure of cerebellar granule cells (7-10 DIV) after in vitro MeHg treatment. In contrast to results in N1818-15 cells, the dihydropyridine nifedipine did not significantly delay the time-to-onset of MeHg-induced elevations in [Ca²⁺], indicating that the routes of Ca²⁺/MeHg entry in the two cell types may differ. Because excitatory amino acids (EAA) receptor-operated channels play a role in excitotoxicity, we hypothesized that granule cells are more susceptible to the effects of MeHg due to the presence of EAA receptor-operated channels. Thus, granule cells were exposed to 0.5 μM MeHg with and without various EAA inhibitors and changes in [Ca²⁺] associated with these interventions were measured using fluo-2. 6-2-2,3,4-2,3,4-Tiodinoiminolinol-2,3,4-HG (DONG, 100 μM), a non-N-methyl-D-aspartate (NMDA) receptor antagonist, inhibited 85% of the kainate-induced elevations in [Ca²⁺], but caused no delay in MeHg-induced [Ca²⁺] elevations. Similarly, 10 μM MK-801 and 100 μM 2-amino-5-phosphonovaleric acid (AP-5), antagonists capable of inhibiting 87% and 82% of the NMDA-induced elevations in [Ca²⁺], respectively, were ineffective at delaying MeHg-induced [Ca²⁺] elevations. These results show that EAA receptor-operated channels do not appear to be the route of MeHg/Ca²⁺ entry in granule cells in vitro. Supported by NIH grant ES03299.

T76.14 DIFFERENTIAL EFFECTS OF Hg²⁺ AND METHYLmercury (MeHg) ON EXCITATORY AND INHIBITORY TRANSMISSION IN HIPPOCAMPAL SLICE. Y. Yuan and W.D. Atchison. Dept. of Pharmacol, Toxicol. and Neurosci. Prgm. Michigan State University, E. Lansing, MI 48824.

Effects of Hg²⁺ and MeHg on resting membrane potentials, excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) were compared in CA1 neurons of rat hippocampal slices using intracellular microelectrode recording techniques. Acute bath application of Hg²⁺ and MeHg (20 or 100 μM) caused biphasic effects on resting membrane potentials, i.e. they initially hyperpolarized and then depolarized CA1 neurons. Hg²⁺ was much more potent and efficacious than MeHg. However, Hg²⁺ appeared less potent in depolarizing hippocampal neuronal membranes than is MeHg. Similarly, Hg²⁺ is less potent at activating GluR1 than MeHg. In contrast, both Hg²⁺ and MeHg blocked IPSPs more rapidly than they blocked EPSPs at similar concentrations. However, single-electrode voltage clamp recordings of IPSCs showed that Hg²⁺ and MeHg had different effects on IPSC decay times and the current-voltage relationships. MeHg suppressed amplitudes of inward and outward currents at all holding potentials and shifted the voltage curve to more positive potentials, i.e. changing the reversal potential from about -75 mV (control) to more positive values. Hg²⁺ also suppressed both inward and outward currents, but it initially caused an increased outward current prior to suppressing it. Moreover, Hg²⁺ appeared to shift the voltage curve to more negative potentials and its overall effects on IPSCs were much slower than those of MeHg. Thus, these results suggest that the mechanisms involved in these actions of Hg²⁺ and MeHg may be different. Supported by NIH grant ES03299.

T76.15 CHRONIC EXPOSURE TO INORGANIC LEAD MODIFIES PKC ACTIVITY IN THE DEVELOPING RAT HIPPOCAMPUS. M. Medlen and V. Miletic. Dept. Corp. Biol. & Environmental Toxicology Center, Univ. Wisconsin, Madison, WI 53706.

The aim of this study was to characterize the subcellular distribution of protein kinase C (PKC) activity in the developing rat hippocampus, and to examine whether chronic lead exposure modifies this distribution. Dams were exposed to either 0 or 100 ppm lead acetate in their drinking water and raised. Offspring were exposed in the first 35 days, and led directly in the drinking water after weaning. The offspring were sacrificed at postnatal days 1, 8, 15, and 29. Blood lead levels were determined at these postnatal periods and correlated to the amount of lead ingested. Total PKC activity was determined in the cytosolic and crude P2 membrane fractions from each animal's hippocampus by an in vitro radiolabeled phosphorylation assay, and was expressed as picomoles of phosphate incorporated per mg of protein per rat. In vivo, total PKC activity in the cytosolic fraction of these normally developing rats increased three-fold between P1 (110 ± 28) and P8 (296 ± 721) (p < 0.0005), remained at these levels at P15, and then decreased somewhat at day 29 (p < 0.05). P2, and PKC activity in the membrane fraction was significantly lower than in control rats at all measured postnatal periods (p < 0.0005). When P8, and P15. These results indicate a significant modification of total PKC activity, especially in synaptic membrane fractions of lead-treated rats. This suggests that the modification may contribute to toxic action in the developing rat hippocampus. (Supported by NIH NS1278.)
Prenatal and Postnatal Chronic Exposure to Inorganic Lead Attenuates LTP in the Adult Rat Hippocampus in Vivo

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We examined whether prenatal and postnatal chronic exposure to inorganic lead modifies the expression of long-term potentiation (LTP) in electrophysiological recordings in the adult rat hippocampus in vivo. pups were given 0, 100, 300, or 1000 ppm lead acetate in their drinking water beginning 2 weeks before birth. Maternal lead acetate was exposed to lead via the milk and, after weaning, in the drinking water. At about 13 weeks of age, a male and a female from each litter were anesthetized with urethane (1-2.5 g/kg), and hippocampal field potentials were recorded. A bipolar stimulating electrode was lowered into the CA3 region, and the glass recording electrode (0.3-2.5MΩ) was positioned in the CA1 region until a maximum response to the CA3 stimulus was observed. LTP was induced by tetanic stimulation (one 400-msec train of five 50-msec pulses at 50 Hz), and recordings of field potentials were repeated at 0.5, 1, 2, and 4 hours post-tetanus. In control and 1000-ppm lead-treated animals (n=3/group) levels of 11.5 ± 0.5 mV increase in the population spike (PS, amplitude of 290-330% above baseline levels) was observed to persist through 4 hours. Animals given 1000-ppm lead (blood levels of 31 ± 2.4 μg/dL) showed little LTP (PS increase <30%). Those exposed to 500 ppm (blood levels of 24.0 ± 4.0 μg/dL) exhibited LTP (200% above baseline) at 1 hour, but then the PS declined to baseline levels. These data indicate that chronic exposure to lead resulting in blood levels as low as 25ug/dL prevents the full expression of LTP in the CA1 region of the rat hippocampus in vivo. The data further suggest that the failure to fully express LTP may contribute to lead's anterograde action in the developing rat hippocampus. (Supported by NIH NS21278).

Effects of In Utero Methymercury Exposure on High and Low Luminance Visual Contrast Sensitivity in Adult Monkeys

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Both animal and human studies have shown that the visual system is sensitive to the effects of methylmercury (MeHg). The present study assessed visual function in adult monkeys (Macaca fascicularis) following in utero MeHg exposure. Maternal exposure of either 0.50, 70, or 90 μg/kg/day of MeHg resulted in blood mercury levels at birth of 1.04 to 2.45 ppm for treated fetuses. There were 10, 9, and 2 offspring produced, respectively. At approximately 9 to 11 years of age, the monkeys were tested on an operant visual psychophysics task designed to assess spatial visual function at low and high luminance. A forced choice task was utilized in which the monkeys faced two oscilliscope screens of equal average luminance and were required to press the button corresponding to the screen displaying a vertical sine wave grating. The following spatial frequencies were assessed: 5.2, 4, 10, 20, and 30 cycles per degree of visual angle for high luminance and 1, 2, and 4 cd for low luminance. Preliminary data from 12 of the monkeys showed that some MeHg monkeys exhibited a decreased contrast sensitivity at high spatial frequency. There appear to be no treatment related effects at low luminance. That some treated individuals appear to exhibit greater visual impairment than others is consistent with previous studies of monkeys exposed to MeHg. These preliminary results, indicate that in utero MeHg exposure may adversely affect the spatial visual function of adult monkeys.

Reduced ChAT mRNA Expression in Septum of Postnatal Rats Following Perinatal Low-Level Lead Exposure:

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We investigated the effect of Pb-exposure on ChAT mRNA expression in postnatal rats. Pups were maternally Pb-exposed by giving 0.25% lead acetate in drinking water to dam. Septal tissue was homogenized in RNAzol B (1:20, v/v). Total RNA from both control and Pb-exposed animals was extracted quantified at OD260, loaded at 20, 10, 5, 2.5, and 1.25 μg onto nylon membrane in Dot-blot manifold apparatus, and hybridized with 32P ATP- radiolabeled 438bp ChAT antisense oligonucleotide probe or 212bp rat 18s rRNA probe, respectively. The blots were scanned and quantified with the aid of a phosphor imager. The mean density of autoradiographic signal was normalized to the corresponding signal obtained with a probe specific for the 18s ribosomal RNA. Relative to control levels, the ChAT mRNA in septa of PN21 and PN28 animals with Pb-exposure were reduced by 42% and 44%, respectively. These results indicate that the presence and expression of the ChAT mRNA following Pb-exposure reflects the reduction of the ChAT mRNA levels. This work was supported by NIEHS grant ES05655.

Chronic Low Level Lead Exposure Alters Calcium Current in Pheochromocytoma (PC12) Cells

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Lead is a known neurotoxin with varied and poorly understood mechanisms of action. We employed the whole-cell patch-clamp technique to examine the chronic effects of low level lead exposure on high-threshold voltage-dependent calcium currents in rat pheochromocytoma (PC12) cells. PC12 cells were exposed to 0, 10, or 50 μM lead-containing growth media for up to 8 weeks. At least four days prior to recording cells were plated in dishes in lead-containing media with nerve growth factor to induce differentiation. As previously reported in acute studies, chronic low level lead exposure caused a dose-dependent decrease in calcium current throughout a 4 week exposure. In contrast, after 5-8 weeks of lead exposure, both the peak and sustained components of the calcium current were increased. When the chronically exposed cells were further challenged with an additional 5 minute exposure to 1 μM lead a dose-dependent decrease in calcium current and an alteration in the activation and inactivation kinetics were observed. The effects of lead were only partially reversible with washing. These data suggest complex interactions of lead with calcium channels in PC12 cells. (Supported by NIH NS21278 & T32 ES07015).

Effects of Intratrually Administered Lead on Extracellular Glutamate and Amino Acid Concentrations in the Striatum: A Microdialysis Study


Previous studies in our laboratory have shown that pretreatment with lead (Pb2+) decreased the basal striatal ECF levels of aspartate (by 50%), glycine (by 18%) and taurine (by 21%), while increasing the K+-evoked release of inhibitory amino acids (glycine and taurine) and decreasing excitatory amino acids (glutamate and aspartate). To further this line of investigation, we measured the ECF amino acid levels of rats intracranially perfused with artificial CSF (aCSF) containing concentrations of Pb2+ (0.1, 0.3, and 0.03 mM) using in vivo microdialysis. Intrahemispheric rats were stereotaxically-implanted with a 3 mm loop-design probe in the striatum and baseline samples collected every 20 min for 2 hours. A Pb2+ containing aCSF (Mg2+ lowered according to preserve ionostomy) was then perfused through the microdialysis probe using a liquid switch for 120 minutes. Compared to controls, aCSF containing Pb2+ (0.1 and 0.3 mM) significantly increased the ECF levels of all amino acids studied, but not in a dose-response manner. The highest concentration of Pb2+ (1.0 mM), where Mg2+ is decreased to 0 mM, significantly decreased the ECF levels of all the studied amino acids compared to controls. These data demonstrate a biphasic dose-response relationship between intratraslal Pb2+ and ECF amino acid levels, suggesting that the observed changes in ECF amino acids may have partly resulted from alterations in aCSF Mg2+ concentrations. Additional studies on the role of Mg2+ and other divalent cations in the release and uptake of amino acid neurotransmitters are warranted.

Effects of Lead on Catecholamine Biosynthesis in PC12 Cells


We have previously reported that lead does not modify dopamine β hydrolase activity, but induces a slight enhancement in tyrosine hydroxylase activity in PC12 cells. We have further studied the effects of lead on a wide range of enzymes in the synthesis and release of dopamine (DA) and norepinephrine (NE).Cells were exposed to 0.3-250μg/ml Pb2+ for up to 3days and the levels of DA, NE and 5HT in the cells determined by HPLC. The release of these amines and their corresponding metabolites was also observed. Pb2+ significantly inhibited the release of NE but not DA. It also reduced metabolic deactivation of DA to 3,4 dihydroxyphenyl acetic acid and homovanillic acid. SHT conversion to 5-hydroxyindolacetic acid was also inhibited. DA and NE storage was only marginally reduced by lead. The inhibitory effects of lead were most prominent after 24hrs exposure and were not significant at 72hrs, suggesting the presence of a tolerance or adaptation process. These results confirm Pb2+ as a potent inhibitor of oxidative metabolic enzyme activity, which could result in increased accumulation of the parent compounds with potential potentiating of their neurotoxicities.

Supported by A

67DR RUS50/ATU98940-02
TT7.1 P300 LATENCY AT CORTICAL SITES IN VISUAL AND AUDITORY ODDBALL PARADIGMS. T.R. Olwek., Department of Psychology, University of Illinois at Urbana-Champaign, Champaign, IL 61820.

P300 latency was mapped during a topographic analysis of normal subjects. The objective was to determine if there is a consistent sequence in which P300 reaches peak amplitude across the cortex. The results indicate that P300 is elicited with both auditory and visual oddball paradigms in ten normal subjects. Peak latencies were found to occur in the sequence in which 10 scalp sites reached maximum amplitude. While individual variation occurred among subjects, several consistencies were seen.

The typical P3-maximum distribution was present along the midline in all conditions. In addition, a large P300-like peak occurred between 240-590 ms at sites away from the midline. This peak reached maximal amplitude at some sites simultaneously, but was delayed as much as 125 ms at other locations. In the auditory condition, the central/parietal regions had the earliest latencies, and the latest latencies were frontal. P300 arose anteriorly in the visual condition, and terminated posteriorly. These results suggest that P300 reaches peak amplitude across the cortex in an orderly sequence. This sequence may provide insight into the cognitive processing associated with P300.


The strategic allocation of attention required to perform difficult cognitive tasks is reflected in corresponding phasic and tonic changes in human brain electrical activity. At present, high resolution electroencephalography (EEG) is the only method of obtaining both sub- and multi-second attentional effects in a single experiment. This may be done by analyzing the data both as discrete averaged Evoked Potentials (EPs), and as continuous unaveraged EEGs. An experiment suitable for both viewpoints was designed around demanding spatial and verbal tasks that required continuous maintenance and updating of representations of working memory (WM). Improved spatial and verbal skills were obtained in EEGs with 115 electrodes, registering the data with anatomical models derived from each (93%) subjects MRI, and correcting for the skull and scalp, in effect estimating the potential distribution just above the cortical surface. For frequent, nontarget stimuli, a positive EP peak at 500ms was larger in the spatial WM condition relative to the other conditions, followed by a peak at 450ms that was larger in both spatial and verbal WM conditions relative to control conditions. The voltage maxima for the P300 and the P400 occurred over dorsolateral frontal cortex near the principal sulci of the right and left hemispheres respectively. These brief (100-200ms) events occurred in parallel with a positive slow wave, maximal over the superior parietal lobe and the supramarginal gyrus, with a slight right-hemisphere predominance. It begins 300ms after stimulus onset, returned to baseline by ~600ms post-stimulus in control conditions, and was sustained for ~1sec longer in the WM conditions than in control conditions. These results suggest that the phasic allocation of attention during WM tasks involves the movemental functional coordination of regions of frontal and parietal cortex. The relationship of these phenomena to multi-second tonic attentional processes is discussed in Part II (Smith & Glucksberg, this issue.).

This research was supported by NSRE AFOB 85, and CNR.


When difficult tasks impose a sustained cognitive load, phasic brain electrical events are accompanied by tonic attention-related events in the ongoing EEG. To characterize these multi-second changes, the data recorded in both focused attention and control conditions were scored into 2 sec epochs corresponding to single trials. Two distinct differences were reliably observed between working memory (WM) and control task conditions. First, a very restricted prefrontal region, power in the 4-7Hz (theta) band, was increased in the WM tasks relative to the control tasks (p < .02). Second, peak power was measured at a midline location over the superior frontal region. Activity recorded in the prefrontal region was unrelated to the source of the signal in or near the anterior cingulate gyrus. Second, over widespread areas of the central and occipital regions, power was more processed over parietal than frontal cortex. A peak power in a 10-12Hz alpha band) also differed between WM and control tasks, with lower power in the WM conditions (p < .001) at a midline location over the posterior margin of the superior parietal lobe. Taken together with the Part I results, these findings suggest that a distributed network of attentional processes, with different timescales in different neuronal populations, are required to maintain attention. It is possible that the effects observed in the tonic region occurred as a result of regional blood flow patterns associated with attentional processing. It is possible that the effects observed in the tonic region occurred as a result of regional blood flow patterns associated with attentional processing. It is possible that the effects observed in the tonic region occurred as a result of regional blood flow patterns associated with attentional processing.

Previous studies have shown that attention to a particular stimulus can increase the activity of the brain area responsible for processing that stimulus. We investigated the effects of selective auditory attention in superior temporal cortex by using functional MRI in 5 young subjects (3M, 2F, 26-27 yrs). Echo-planar images, using a 1.5 Tesla GE scanner, were obtained while subjects listened to 3 word lists. Subjects heard each list three times (for a total of 9 presentations with three periods of no stimulation, each 30 sec in length, and the order of presentation of the words lists was varied across subjects). Images obtained during stimulation were compared to those obtained during rest using an ANOVA (p<0.01) with a subsequent test of significance on the spatial extent of each cluster of activated voxels (p<0.01).

All subjects showed significant areas of activation in left auditory cortex during all listening conditions compared to rest. Right hemisphere activation also was in 2 subjects scanned with a full-head coil. Three of the five subjects showed a mean activation during the attention conditions that was substantially larger in spatial extent than that seen during the passive listening condition (12%, 34%, and 338%). Two subjects had an equivalent number of voxels activated in the attention and passive conditions. These results provide preliminary evidence in support of an attention-related enhancement of activation in auditory cortex.

PET STUDY OF AUDITORY AND VISUAL ATTENTION. D.S. O'Leary, N.C. Andreasen, R. Hurtig, L. Fleshman, I. Torres, R. Hitchwa, Mental Health Clinical Research Center, University of Iowa, Iowa City, IA 52242.

We have previously found that attending right or left for dichotically presented stimuli caused asymmetric changes in rCBF in auditory cortices (O'Leary et al., Brain and Language, in press). The present study used simultaneously-presented visual and auditory stimuli to evaluate modality-specific spatial attention. During different conditions of a PET study with (O-15) water, 13 normal volunteers attended left and right to visually- or auditory-presented consonant-vowel-consonant (CVCC) sequences. During visual conditions, subjects fixated centrally, but monitored right or left visual fields while ignoring dichotic stimuli. Attention to one or the other visual fields caused asymmetric changes in rCBF in extrastriate cortices, but did not induce asymmetric rCBF changes in auditory cortices. The activation in auditory cortices resulting from nonattended dichotic stimuli was asymmetrically symmetric. When subjects attended to dichotic stimuli, we replicated our finding that attending left or right caused asymmetric rCBF in temporal lobes, but found that rCBF in occipital cortex was unaffected. The pattern that spatial attention is modality-specific. Regions of interest were traced on each individual's MR images which were co-registered with their PET images, allowing measurement of rCBF in Heschl's gyrus, the planum temporale, the ascending rami of the Sylvian fissure and striate cortex. This data, which will be discussed, address the anatomical location at which attentional effects occur in sensory systems (i.e., primary vs secondary cortices).

PET STUDY OF AUDITORY AND VISUAL ATTENTION. D.S. O'Leary, N.C. Andreasen, R. Hurtig, L. Fleshman, I. Torres, R. Hitchwa, Mental Health Clinical Research Center, University of Iowa, Iowa City, IA 52242.

ATTENTION MODULATES IMRI ACTIVATION IN HUMAN MTR. J. Kentros, R. Spreng, J. Krienen, D. Rossoe, I. Rossoe.

The Rowland Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA, 02142. 1GMH-NMR Center, 149 13th St., Charlestown, MA 02129. (e-mail: Kentros@med.harvard.edu).

We used functional MRI to measure the amount of activation within functionally defined regions of the human brain homologous to monkey area MT and MST. Subjects viewed a sequence of both moving and stationary dots. Motion was radial, and subjects fixated a central fixation point. Initially, subjects attended to the moving dots and ignored the stationary dots. Every 20 sec subjects were cued to voluntarily switch attention between the moving dots and the stationary dots. The magnitude of the fMRI signal was significantly increased when subjects attended to the moving dots than when they attended to stationary dots and ignored the moving dots.

Additional experiment was quantified to more precisely the modulation just described. Subjects viewed the moving-and-stationary-dot stimulus during their 20 second epochs, alternating with epochs of a stimulus in which only stationary dots were presented. During the 20 seconds when the stationary and stationary-dots, subjects attended to the moving dots, and during the other two epochs, they attended to the stationary dots. Attending to stationary dots did not eliminate MT activation. Rather, MT was highly active any time a stimulus containing visual motion was present. However, when attention was directed to the stationary dots, activity was only 80-90% as strong as when attention was directed to the moving dots. This attentional modulation was significant (p<.001) for each of three subjects.

What does the modulation of MT activation represent? Is it additional activation during the "attend moving" condition, or is it suppressed activation during the "attend stationary" condition, because the moving dots need to be ignored? Experiments are underway to examine this question.

FUNCTIONAL MRI OF AUDITORY EFFORTFUL ATTENTION IN HUMANS. I. Deutsch, J. Langendorff, B. Woldorff. EEG Lab, Hebrew Medical School and MGH-NMR Center, Charlestown, MA 02129.

Attention becomes effortless with increased working memory load, needs for interference control, or difficulty of target detection. Using functional MRI (fMRI), we investigated if an auditory effortful attention task would activate regions reported for visual selective attention and thalamus. Seven normal volunteers had contiguous 7mm axial images covering pons to parietal cortex acquired with an asymmetric spin-echo instantaneous sequence. The paradigm employed an A-B-A-B design with stimulus blocks of 90 letters presented one letter per second. In A, subjects responded to the letter "a" when immediately preceded by a "g" (QA). B conditions required the subject to respond to the letter "a" when preceded by the QA letters (p=0.04). ABAQA, and ignoring embedded false cues and targets. Target probability was matched between QA and QJA conditions at 2%. Subjects were scanned three times to correct for intersubject variability. Data were examined using three novel averaging techniques and nonparametric statistical mapping. Comparison to QA, the QA condition produced the most robust effects, but QA effects were only revealed in the parietal-occipital cortex (PCP, thalamus, superior colliculus (SC), supplementary motor area (SMA), frontal eye fields (FEF) and supplementary eye fields, and ventrolateral prefrontal cortex, and selectively in right inferior temporal cortex (ITC). Significant negative signal change occurred in the anterior cingulate (AC). This study found activation in regions proposed by Posner and Cohen to form a posterior network for orienting to sensory events (i.e., PPC, thalamus, and SC), along with regions forming an anterior network for signal detection (i.e., AC, SMA). Our auditory task also produced significant rCBF in lIPC, SC, fFEF, and rFEF. Our results may result in cognitive strategy employed. Effortful attention appears to depend on the entire distributed network implicated for selective attention, with important components being independent of sensory modality of stimuli.
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779.11

SEQUENTIAL EXPRESSION OF TRK A, B AND C DURING DEVELOPMENT AND REGENERATION OF OLFACTORY RECEPTOR NEURONS: A. Jane L. Roskams*; Angelyn Bethel; Laurie Williams and Gabriele V. Ronnett. Department of Neuroscience and Neurology, Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore MD 21205.

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins -3,4 and 5 (NT-3,4,5) have been identified and characterized by their ability to induce neuronal differentiation and support neuronal survival in vivo and in vitro. A family of neurotrophin receptor tyrosine kinases (Trks) mediates neurotrophin action and neurotrophin binding has been shown to stimulate homoregulation of one Trk. Heterodimerization of two Trks or one Trk and the low affinity NGF receptor (LNGFR). Trk A, B, C and the LNGFR are expressed in adult and developing olfactory neuroepithelium. The olfactory neuronal epithelium is capable of undergoing neuronal regeneration throughout the lifetime of the organism. When the olfactory bulb is removed, the neuroepithelium enters a state of coordinate-regeneration, where there is a sequential expression of Trk A (precursor neurons), B (immature neurons) and C (mature neurons) within regenerating olfactory neurons at different stages of differentiation. In primary cultures of olfactory neurons Trks become transcriptionally expressed when challenged with the appropriate neurotrophin ligand and activate a signal transduction cascade that terminates in the up-regulation of immediate early genes such as fos and jun. We are currently examining the olfactory pathways of mice with a null mutation for the Trk A, B and C genes to determine whether the phenotype of these knockout mice supports the suggested in vivo role for each Trk and to examine the possibility of neurotrophin receptor redundancy during development in this system.

780.3

DYNAMICS OF TRK4 PHOSPHORYLATION IN RESPONSE TO INTRACRABEGAL NEUROGEN INJECTIONS IN RODENTS. L.F. Kromer,* and D.R. Kaplan. Dept of Otolaryngology-Head & Neck Surgery, Georgetown University Medical Center, Washington, DC 20007 and the National Cancer Institute, Frederick, MD 21702.

Cholinergic innervation in both the septum/parabrown forebrain and neostriatum are responsive to nerve growth factor (NGF) and express trkA receptors, the high affinity receptor for NGF. Although PC12 cells have been extensively used to study NGF induced trkA phosphorylation and subsequent signal transduction in vitro, little is currently known about NGF induced signaling through trkA receptors in vivo. Thus, the present experiments were designed to evaluate the dose response and time course of NGF induced trkA phosphorylation in both the septum/parabrown forebrain and neostriatum (NS) after intraventricular (ICV) injections of NGF in adult rats. ICV injections of NGF exhibit a clear dose response (10ng, 100ng, and 1ug) in the degree of receptor phosphorylation in both the septum and NS. Moreover, a single ICV injection of NGF (1 pg/ug) results in a prolonged receptor activation in the septum and NS that can be detected by 30 min., is maximal from 2-12 hrs., and decreases to baseline levels by 24-36 hrs. To determine whether the dynamics of this response could be altered, multiple ICV injections of NGF were administered. When 3 ICV injections of NGF were administered at 48 hr intervals, there is a prolonged activation of trkA receptors in both the septum and NS which lasts for at least 72 hrs. This potentiation of receptor phosphorylation with multiple NGF injections has important implications for the design of possible CNS treatment therapies with neurotrophic factors. Supported by NIH grant NS-31445.

It has been proposed that the functional high-affinity nerve growth factor receptor is a heterodimer of two proteins gp75 and TrkA. We have obtained direct evidence of the existence of this complex by a combined approach of fluorescence recovery after photobleaching and fluorescence confocal microscopy, and site-directed mutagenesis. When gp75 is expressed alone in either mammalian PC12 cells or using baculovirus-infected insect S99 cells, it is highly mobile as determined by FRAP. Coprecipitation with TrkA causes a reduction in the mobile fraction. The immunoactivity of gp75 is not observed, if the cytoplasmic domain of gp75 is truncated or if TrkA is mutated to inactivate the ty-kinase domain. Analysis of diffusion coefficients in the presence and absence of NGF suggests that gp75 and TrkA are complexed, even for mutated receptors which do not bind NGF with high-affinity. Direct evidence of gp75-TrkA complex was obtained by copatching studies. It was found that patching of gp75 by crosslinking antibodies resulted in copatching of TrkA. Analysis of mutated receptors by copatching indicates receptor complexes in the absence of high-affinity NGF binding sites. Using chimeric receptors of TrkA and Trk, we determined that the extracellular domain of TrkA is critical for efficient copatching. We suggest that extracellular interactions drive complex formation, but functional high-affinity NGF binding results from a conformational change involving intracellular and extracellular domains of both receptors.

DIFFERENTIAL DEVELOPMENTAL EXPRESSION OF THE NEUROTROPHIN-3 RECEPTOR (TrkC) ISOSFORMS IN THE ENTERIC NERVOUS SYSTEM. J. D. Javaheri, J. A. Davidson, D. A. Davies and L. M. Kaplan. * Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114.

cDNA cloning studies have suggested the existence of multiple, neurotrophin-3 (NT-3) receptor isoforms encoded by the trkC gene. In the rat, alternative splicing of the pre-mRNA transcript gives rise to a “kinase competent” isoforms that encode receptors varying by the presence or absence of 14, 25, or 39 amino acid carboxyl terminal domains, respectively (C4, C4-C5, and C5). Four additional “truncated” TrkC isoforms, with identical extracellular ligand-binding domains, contain unique intracellular sequences in place of the kinase domain (TrkC 14, TrkC 25, TrkC 39, and TrkC 158). We have developed two reverse transcription polymerase chain reaction (RT-PCR) based strategies for the specific expression of the various trkC isoforms. First, quantitative PCR can be easily detected, competitive mutant construct is used to quantify total trkC expression. Primers flanking the kinase domain splice site competitively amplify the four kinase competent isoforms, allowing measurement of the relative expression of these mRNAs. The second strategy uses techniques to measure expression of mRNAs encoding the truncated receptors. These studies reveal that C5 and C4 are the predominant kinase competent isoforms and TrkC 14 is the predominant truncated isoform in peripheral tissues and within the gastrointestinal tract. C4, C5, and TrkC 158 are expressed at lower levels in selected tissues. Isoform expression is strongly regulated during the first stage of intestinal development with selective increases in the expression of the C4, C5 and C4 isoforms. The regulated expression of these functionally distinct isoforms suggests that response to NT-3 is determined by the developmental and physiological state of the target neuron.
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781.1 MEMBRANE-DELIMITED MODULATION OF NMDA RECEPTOR CHANNELS BY METABOTROPIC GLUTAMATE RECEPTORS VIA PERTURBATION OF CYTOSKELETAL NEURONS. S.P. Yu, D.M. Turetsky and D.W. Choi, Department of Neurology and Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

The excitatory neurotransmitter glutamate activates both ionotropic and metabotropic receptors (mGluRs). mGluR activation modulates many neuronal mechanisms, including the behavior of ionotropic NMDA, or AMPA/kainate receptors, usually by inducing changes in the levels of the second messengers, IP3 or Ca2+. Using whole cell and single channel recordings, we studied the modulation of NMDA receptor currents by mGluRs on mouse cortical neurons. The mGluR agonists 1S,3R-ACPD (200 μM), L-CCG-I (100 μM), (S)-3-hydroxyethylpropionate (3HPP, 100 μM) and (S)-carboxy-bis(3-hydroxypropionate (4CHP, 100 μM) attenuated the NMDA receptor-mediated whole cell current by 20-80%. However, bath-applied 3HPP (mGluR 1/2 agonist) and 4CHP (mGluR 2/3 agonist) showed no effect on NMDA single channel activity in cell-attached recordings. In isolated outsole patches, 20-100 μM 3HPP but not 4CHP decreased the open probability (Popen) of NMDA receptor channels. This modulation was blocked by the mGluR antagonist, (R)-3-(6-carboxy-4-aminopentyl)glycine (MCPC, 500 μM), as well as GYPS (200 μM), but not by permus toxins (TX, 0.2μg/ml for 20-24 h). 3HPP itself is unlikely a NMDA receptor agonist since, in HEK 293 cells lacking endogenous mGluRs, it did not affect single channel currents mediated by cloned NMDAR1/2A receptors. Present results thus provide novel evidence that a glutamate receptor 1/2 agonist may interact locally with NMDA receptors via PTX-inactivating G proteins, without the involvement of readily diffusible messengers. This modulation may have important implications for synaptic plasticity or neurodegeneration. Supported by NS 30337 from NINDS (DWC).


Ionotropic glutamate receptors (GlRs) are neurotransmitter-activated ion channels that mediate excitatory synaptic transmission in the CNS. These membrane receptors are composed of multiple subunits organized around a central pore. Their subunit stoichiometry remains unknown. In analogy to the nicotinic cholinergic receptor (AChR), the prototype of ligand-gated ion channel, GlRs are considered to be pentamers. Recent data, however, suggest that GlRs and AChRs may have different protein topologies. This raises the question: are GlRs pentameric assemblies? To address this issue we used a functional differential sensitivity assay1 based on the differential sensitivity of two GlR1 mutant subunits to open channel blockers such as PCTP and MK-801. Conjection into Xenopus oocytes of weakly-sensitive subunits with highly-sensitive mutant subunits produced functional receptor channels with mixed drug sensitivities. Augmenting the fraction of the weakly-sensitive subunit in the mixture increases the affinity of the complex for the channel blockers. Assuming that both classes of mutant subunits aggregate randomly, the subunit stoichiometry of GlR1 was determined to be pentameric. Then finding that GlRs are pentamers is compatible with a conserved subunit stoichiometry for the members of the ligand-gated ion channel superfamily.


781.4 DETERMINATION OF GLYCINE BINDING SITES IN THE M3-M4 LINKER OF THE NMDA RECEPTOR NR1 SUBUNIT. M. Wooll, H.K. VanDoom, and A.M. VanDoo1. Department of Pharmacology, Duke Univ., Durham, NC 27710, USA.

Initial topology models for ligand gated ion channels included four putative transmembrane domains (M1-M4). The ionotropic glutamate receptors are a subfamily of these channels for which the four transmembrane domain model has recently been rejected. NMDA receptors are a glutamate receptor subtype comprised of two subunits (NR1 and NR2). By manipulating N-glycosylation sites in the NR1 subunit, we have localized the extracellular portions of the M3-M4 linkers to the extracellular space. Introduction of a novel glycosylation site immediately past M3 resulted in a functional channel displaying a fractional response to glycine, consistent only contaminating amounts of the agonist glycine. Attempts to map the M3-M4 linker region in other glutamate receptors have resulted in non-functional mutants. Despite the inability to functionally verify peptide prowess, it has been concluded that the entire M3-M4 linker exists in the extracellular space. Inconsistent with this conclusion, however, is the prior determination of a functional PRA phosphorylation site in the M3-M4 linker of GlR1. In order to test for the possibility of an intracellular region within the M3-M4 linker, we introduced a canonical PRA phosphorylation consensus sequence (RRASL) into the NR1 subunit. This mutation reduced the apparent affinity for glycine by more that a magnitude without affecting the apparent affinity for glutamate. The EC50 for the competitive glycine antagonist, 7,5-dichlorokynurenamine (DCK) measured at the EC50 for glycine was not different. These results suggest that the glycine binding site in GlR1 is mapped with these two additional markers.

780.11 INSULIN IMMUNOPRECIPITATION OF P85a 5-HT1a ACTIVATED PROTEIN (INK) KINASE IN COLOURED FISH MESDORS. L. Barres, and P.A. Paulinom. Department of Pharmacology, UCL, and the Department of Pathology, CB0, 2012.

The NRK kinase cascade involving ERK2 is a major signaling system by which cells transduce extracellular stimuli into intracellular responses. Recently, 2 other NRK kinase cascades, involving JNK/SAPK and p38/NR activity, have been discovered. Little is known about the regulation of these two NRAs by P85b receptor tyrosine kinase. In this study, we examined the MAP kinase present in electric fish forebrain homogenate by means of an immunoprecipitation with a specific NRK antibody for these cells. Neuronal cell lines synthesize chromatin on Hg and assayed with myelin basic protein (MBP) showed peaks of protein kinase activity eluting at 30 (MK1) (Peak 1), 170 (MK1) (Peak 2), and 420 (MK3) (Peak 3). Peak 1 contained NRI, asayed with a GST-c-Jun fusion protein as substrate, and was identified as MK1. Peak 2 and NRK2 (identified with ERK1 and MK2-C-terminal antibodies). Insulin (50 ng/ml, 15 min) had no significant effect on JNK or ERK2 activity but markedly inhibited the 3rd peak of kinase activity by 25-30%. Analysis of Peak 3 by mass-precipitated gel assays revealed a 38 kDa kinase. Like other MAP kinases, the p38 kinase is abundant in neurosecretory neurons. The p38 kinase is abundant in neurons. The p38 kinase is abundant in neurons. The p38 kinase is abundant in neurons.

Five subunits, GluR5, GluR6, GluR7, KA-1 and KA-2, belonging to the ionotropic glutamate receptor family of the kainate type have been cloned. Glutamate receptor transcripts, including those for kainate, showing a receptor-specific response, but not by AMPA, have been described in cultured hippocampal neurons. To determine the kainate receptor subunits which take part of native kainate receptors, we have applied a multiplex polymerase chain reaction of cDNAs reverse transcribed from mRNA harvested from single cultured hippocampal neurons after electrophysiological recording. We found that all the cells showing rapidly desensitizing currents in response to kainate express the GluR5 subunit mRNA and that some of them also express the GluR6 subunit mRNA. No GluR7, KA-1 or KA-2 mRNA was detected. Analysis of the editing sites of the GluR6 mRNA demonstrated that the editing sites present in these subunits are edited to a different extent, and that the GluR5 site from the GluR6 subunit controls functional properties of native kainate receptors, similar to recombinantly expressed homomeric GluR5 receptors.

871.6 VISUALIZATION OF GLUTAMATE-GATED CHANNEL PERMEATION: HIGH RESOLUTION MAPPING OF NEURONAL SUBPOPULATIONS. B. E. Magee, Moran Eye Center, University of Utah, Salt Lake City, UT 84102.

Very few retinal neurons possess glutamate (Glu) receptors but the partitioning of AMPA/kainate (AMPA/KA) and NMDA subtypes is known for a but of over a few of known cell types. Most Glu-gated cation channels are permeable to guanidinium* derivatives, including 4-aminoguanidinobutate (AGB), and Glu-gated AGB channel permeation can be visualized by anti-guanylation antibodies. Objective: Map Glu-gated AGB permeation in all retinal neurons. Methods: Isolated goldfish retinas were challenged 15 min in vitro with Glu agonists / antagonists and 25 mM AGB substituted for Na*, then analyzed with multispot protocols for deriving quantitative amino acid and AGB immunoreactivities. Results: All horizontal cell types, all identifiable OFF center bipolar cells (BCs), half of all GABAergic amacrine cells (Acs) and all but one glycinergic AC type demonstrated graded, CNOX-blocked AGB permeation gated by KA (0.125 µM) but no detectable signal after NMDA (3-3000 µM) exposure with or without Na2. In particular, the unique cholinergic/GABAergic ACs showed that antagonist AGB/gated AGB agonists alone. On center BCs and solitary population of glycinergic Acs possess neither AMPA/KA: nor NMDA-gated responses. A large subset of GABAergic Acs displayed strong responses to both KA and NMDA. NMDA responses were blocked by AP-7 and MK-801. Most ganglion cells appear to bear both types of receptors although a small set seems driven exclusively by AMPA/KA systems. Conclusions: AGB permeation is a powerful tool for partitioning the Glu-gated receptors of complex neuron populations. There are three forms of Glu permeation in retinal neurons: (1) Pure AMPA/KA systems; (2) Dual NMDA and AMPA/KA systems; (3) Those with neither. Support: NIH EY02372 and a Jules and Doreen Stein Research to Prevent Blindness Professorship.

871.7 TRANSIENT AND PERSISTENT PHOSPHORYLATIONS OF AMPA-TYPE GLUTAMATE RECEPTOR SUBUNITS IN CEREBELLAR PURKINJE CELLS. K. Nakazawa* S. Miyata*, T. Hashikawa* and M. It* Lab. for Synaptic Function and 'Neural Systems, Frontier Res. Prgrm., The Inst. of Phys. and Chem. Res. (RIKEN), Saitama 351-01, Japan

Ionotropic glutamate receptor (GluR) phosphorylation is postulated to play a role in receptor desensitization and long-term synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD). To investigate the relationship of AMPA receptor phosphorylation with synaptic plasticity, we developed a method for detection of a phosphorylation peptide containing Ser-662 (that is a mononclonal antibody named 9Pb) or Ser-662 (a polyclonal antibody named 12Pb) of rat GluR2. Immunoblot analysis showed that antibodies recognized subtypes of cerebellar AMPA receptor subunits, which were phosphorylated. 12Pb-immunocytochemistry revealed that a brief exposure of a rat cerebellar slice to AMPA leads to transient phosphorylation of the AMPA receptor subunits in Purkinje cell dendrites, providing evidence of activity-dependent receptor phosphorylation in a cell-type specific manner. An immunoelectronmicroscopic analysis showed that postsynaptic AMPA receptors at parallel fiber-Purkinje cell synapses were phosphorylated following AMPA application. Furthermore, persistent phosphorylation over 30 min was obtained when the exposure to AMPA was preceded by a 15 min perfusion of the slice with 8-bromo-cyclic GMP, dibutyryl-cyclic GMP or calcium A., the stimuli causing long-term desensitization of Purkinje cell AMPA receptors. In contrast, marked 9Pb immunolabeling was observed in Bergmann glial cells regardless of stimulai causing LTD. These findings support the view that phosphorylation of GluR channels is of critical importance in the regulation of synaptic efficacy. Although our present results are inconsistent with three transmembrane model of GluR, our intracellular receptor phosphorylation might be explained if GluR membrane topology is different in a cell-type and/or subunit specific manner. Alternatively, it may be changed dynamically according to synaptic activity.

871.8 SINGLE-CHANNEL ANALYSIS OF A HIGH AFFINITY Ca"' AND Mg"' BINDING SITE IN THE "POLE OF WIRE" MODEL OF THE RECOMBINANT NMDA RECEPTORS. A. Arayush* and L. Premkumar. Dep. Biophysics, SUNY at Buffalo, Buffalo NY 14214.

Recently the "QRF" site in the "pole of wire" model of the glutamate receptor channel was proposed to be a high affinity site for Ca++ ions. We have analyzed the Ca++ occupancy of the recombinant NMDA or AP5 receptors in Xenopus oocytes, using single-channel analysis. The Ca++ occupancy was determined using non-radioactive Ca++ ionophores and the computer program of Lloyd et al. (Nature, 1988). The Ca++ occupancy of the QRF site was determined by using the non-radioactive Ca++ ionophore A23187 and the computer program of Comer et al. (Proc. Natl. Acad. Sci., USA, 1987). The Ca++ occupancy of the QRF site was found to be about 40% in the absence of Mg++. In the presence of Mg++ the Ca++ occupancy of the QRF site was found to be about 60%. These results are consistent with the "pole of wire" model of the glutamate receptor channel.
EVIDENCE FOR MULTIPLE AMPA RECEPTOR COMPLEXES IN PYRAMIDAL NEURONS OF THE CA1/CA2 REGIONS OF THE HIPPOCAMPUS. R.J. Wenhao, A.R. Niedzwiecki (1), J.R. Blanks, and J.E. Portela. Laboratory of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

The four AMPA receptors form functional homomeric or heteromeric receptor complexes when expressed in vivo. Based on their physiological properties, it is thought that receptor complexes in neurons are homomeric, containing one or two different subunits. While most neurons express multiple AMPA receptor subunits, it has not been determined if the complex form is functional or inactive. The type of the neuron is capable of forming multiple complexes which differ in their subunit compositions, and therefore, their functional properties. Hippocampal CA1/CA2 pyramidal neurons are an ideal model system for studying the composition of AMPA receptors in a neuronal population. These neurons are relatively homogenous in their functional properties and their abundant expression of Glur1, 2, 3, and a relatively pure preparation of the cell bodies and their dendrites can be obtained by dissection. Receptors were solubilized with Triton X-100. Western blots confirmed abundant Glur1, 2, 3 and low amounts of Glur4.

GluR2/3 immunoreactivity was shown to be present in CA1/CA2 neurons, those made up of Glur1/Glur2 and those made up of Glur2/Glur3. A population of homomeric Glur1 is also present.

INSULIN POTENTIATION OF NMDA RECEPTOR CURRENTS IN Xenopus OOCYTES: EFFECTS OF TYROSINE KINASES. S. Chen and J.P. Leonard. Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607.

Insulin receptors are present in mammalian brains as well as in Xenopus oocytes. As little as 80 nM insulin for 10 min potentiates the NMDA-activated currents on expression of heteromeric (1/4+1) receptors in Xenopus oocytes. A 100 nM insulin concentration of 0.4 µM insulin potentiated to 15% of control (157 ± 5%, n=37) reaching a maximum at 30 min. To test whether insulin potentiation was due to activation of tyrosine kinase, oocytes pre-treated for 30 min with 0.8 µM insulin were incubated in 100 µM genistein, an inhibitor of many tyrosine kinases. The insulin receptor potentiation was reversed by genistein, but not by 100 µM daldezin, the inactive analog of genistein. We also injected exogenous tyrosine kinase pp60src into Xenopus oocytes expressing (1/4 receptors and found pp60src could potentiate NMDA receptor currents also (to 140 ± 7%, n=12). Both evidence indicates that tyrosine kinases are involved in potentiation of heteromeric NMDA receptor. Genistein also inhibited the NMDA-activated currents in oocytes (to 81 ± 9% of control), n=4) in 30 min without insulin treatment. This suggests that there is basal tyrosine kinase activity in oocytes and that insulin stimulates this tyrosine kinase activity to potentiate NMDA receptor currents.


Synaptophysin (SN) is a glycoprotein found in vesicles of mature synapses, and is considered a marker for the presence of synaptic terminals. The pituitary gland of mammals is innervated by nerve fibers of hypothalamic origin which enter both the neural (NL) and intermediate (IL) lobes, with some studies showing anterior (AL) lobe innervation. Previous studies have shown reduced tyrosine hydroxylase and GAP-43 immunoreactivity in the aging pituitary. We examined the glada of younger and aged rats for SN-19 to determine if relative numbers of synaptic terminals are altered with aging. Adult male Sprague-Dawley rats, ages 8, 13 and 15-17 months were halothane anesthetized, perfused intracardially with buffered paraformaldehyde, and paraffin sections of pituitary tissue were immunostained for SN, using a monoclonal antibody (dilution 1:50) from Boehringer-Mannheim. Fine, punctate staining surrounded endocrine cells in the IL, with dense staining a number of GAP-43-LI fascicles could be seen. Also present were individual varicoses fibers. They were distributed mainly among the gland cells, although some could be found along the blood vessels. We examined the effect of aging on synaptophysin immunoreactivity in the anterior pituitary. Particularly striking was the dramatic increase in the immunoreactivity among the gland cells. Large numbers of GAP-43-LI varicosities of different sizes, many fairly thick, could be seen to gather around clusters of the gland cells, seemingly in close contact with them. The results imply an active axonal sprouting following this hormone manipulation and strongly support our hypothesis of neural-humoral dual regulation of the mammals anterior pituitary.

In vitro studies using various cellular systems have provided conflicting results regarding the regulation of somatostatin receptor (SSTR) expression. In vivo studies in rodents show that certain somatostatin receptors (SSTRs) are downregulated in response to ghrelin (GHRH). This study aimed to investigate the effects of ghrelin on the regulation of SSTR expression in vivo.

Methods: Male Wistar rats were divided into two groups: control and ghrelin-treated. The ghrelin-treated group received daily injections of ghrelin (60 ng/kg/day) for 7 days. Tissues were collected and subjected to immunohistochemistry for SSTR expression. The density of SSTR-positive cells was quantified.

Results: Ghrelin significantly decreased the density of SSTR-positive cells in the hypothalamic paraventricular nuclei (PVN), arcuate nucleus (ARC), and lateral hypothalamus (LH). No changes were observed in the medial basal hypothalamus (MBH) or the ventromedial hypothalamus (VMH).

Conclusion: GHRP-6, the ghrelin receptor agonist, blunts the SSTR expression response to ghrelin, suggesting a potential role for SSTRs in the regulation of ghrelin secretion and ghrelin-induced SSTR expression.
782.0 T

MODULATION OF CORTICOTROPIN-RELLEASING HORMONE RECEPTOR EXPRESSION IN HUMAN PINOBLASTOMAS BY ACTH: A PERSPECTIVE FROM THE PERSPECTIVE OF THE HUMAN PINEAL GLAND

S. R. Wray, R. L. Lerner, and D. R. Ralls

The human pineal gland is a major source of melatonin, which is involved in the regulation of circadian rhythms. Recent studies have suggested that the pineal gland may also play a role in the regulation of other hormone systems, including those involved in the stress response. In this study, we examined the expression of corticotropin-releasing hormone (CRH) receptor mRNA in human pineal tumors and correlated this with the expression of ACTH receptors.

782.1 T

FLUORESCENCE IMAGING OF INTRACELLULAR SODIUM IN RAT HIPPOCAMPAL ASTROCYTES

C. Rose, B. R. Ransom, and D. J. Kandel

Sodium is an important ion for maintaining cell excitability and for the regulation of osmotic balance. In this study, we used fluorescent techniques to visualize intracellular sodium (Na+) in hippocampal astrocytes. We found that Na+ levels were highest in the cell bodies of astrocytes and lowest in the processes, consistent with the hypothesis that astrocytes regulate extracellular Na+ levels.

782.2 T

INFLUENCE OF K+ AND CL- CHANNELS IN MICROGLIA FUNCTION

L. Schlichter, B. Belyi, C. Sokoloff, P. S. Penner, and D. J. Phillips

Microglia are a type of immune cell that plays a role in neuroinflammation. In this study, we investigated the role of K+ and Cl- channels in microglia function. We found that blocking these channels altered microglia morphology and increased their phagocytic activity.

782.3 T

NEUROENDOCRINE REGULATION: OTHER III

782.3.0 ORIGIN OF CORTICOTROPIN-RELLEASING HORMONE INNERNATION OF THE SHEEP ADRENAL DURING FETAL, NEONATAL AND ADULT LIFE

T. McDonald, N. W. N. Nathans, and J. S. Foy

Corticotropin-releasing hormone (CRH) is a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis. In this study, we investigated the origin of CRH in the sheep adrenal during fetal, neonatal, and adult life. We found that CRH is initially produced by fetal adrenal cells, and that CRH production decreases during the perinatal period.

782.3.1 MODULATION OF PINEAL FUNCTION BY ACTH: A PERSPECTIVE FROM THE PERSPECTIVE OF THE HUMAN PINEAL GLAND

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THURSDAY AM

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995

ION CHANNELS: CELL FUNCTION III

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ABNORMALITY IN THE ACTIVATION OF Ca-ACTIVATED POTASSIUM CHANNELS REVEALED IN STUDIES OF NEUROFIBROMIN-DEFICIENT PC12 CELLS. R. L. 8. Sanders* and Elaine Lengyel**, Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908 & Brown University, Providence, RI 02912

Individuals with Neurofibromatosis-1 (NF1) manifest electrophysiological abnormalities in transmission of nerve impulses along both peripheral and auditory nerves. As a first step toward understanding the biology of this neuronal signaling, we have used whole cell and single channel recording to characterize the ionic currents expressed in neurofibromin-deficient PC12 cells. We report that NF1-deficient PC12 cells have a significantly altered ionic profile compared to their wild-type counterpart. The most prominent difference from wild-type cells was the complete lack of Beroepos-sensitive maxi-K-current in 7/7 whole cell recordings from NF1-deficient PC12 cells. Concomitantly, there was no KCa channel activity in single-channel recordings from patches of NF1-deficient PC12 cells in the cell-attached mode. However, the addition of PM Ca to the cytosolic surface of excised patches induced the activation of high conductance KCa channels, suggesting that the channel is expressed basally inactive. We detected an RNA species of ~6.5 kb that hybridized to a slow-potential DNA probe on Northern blots of total RNA from both mutant and wild-type cells, confirming Kc expression. Thus, our results suggest an uncoupling, in NF-deficient cells, of maxi-K channels from the supply of calcium normally required for activation. We are now focusing our studies on voltage-gated calcium channels, which, orally provide the main route of calcium entry for activation of the maxi-K during excitation. Our preliminary studies indicate that voltage-gated calcium channels are also expressed in NF-deficient PC12 cells and that there is a deficit in the mechanisms which govern Kc/CA channel co-localization. We are also testing the hypothesis that these changes, in NF-deficient PC12 cells, are a direct consequence of mutations in the NFI locus by comparing the ionic properties of neurons from NFI homozygous and heterozygous mutant and wildtype mice.
784.1
CHARACTERIZATION OF MULTIPLE OPIOD BINDING SITES WITH [3H]HUPHENORPHINE IN RAT BRAIN MEMBRANES
1Department of Pharmacology, and Isotope Laboratory, Biological Research Center, Hungarian Academy of Sciences, H-1171 Szeged, P.O. Box 521, and 2) ALKALOIDA Chemical Works, H-4440 Tiszavari, P.O. B. 1, Hungary

Huphenorine (Tongue®) was a potent and selective ligand at the positions 15 and 16. The purified end-product has 2.35 TBQ/mmol (63 Ci/mmol) specific activity. The radioactive label was stable under acidic conditions, but not in the presence of strong base. Binding characteristics of [3H]Huphenorine were evaluated in particulate membrane fractions of rat brain. Specific ligand binding was of high affinity, saturable and naloxone-sensitive i.e. opioid in nature. The binding interaction displayed reversibility, and high degree of stereospecificity as measured by chiral selective opioid compounds. In kinetic studies, equilibrium binding was achieved in 40 min incubation at room temperature, and dissociation of the receptor-ligand complex occurred steadily when initiated by the addition of unlabelled opioid ligands. Homologous competition experiments reveal that [3H]Huphenorine binds to an apparently single set of binding sites with a Kd value of 2.1 ± 0.4 nM. The maximal number of these sites was found to be 1.35 ± 0.27 pmol/mg protein. The binding parameters were not significantly changed in the presence of 100 mM sodium ion confirming the mixed agonist-antagonist properties of huphenorine. In rat brain the ligand showed a relative preference for i and kappa binding sites although opioid delta sites were found also able to be labelled. The radioligand is capable of labelling kappa opioid receptors in frog brain membranes and is a promising tool for studying ligand - receptor interaction in the opioid system.

784.3
INHIBITION OF L-TYPE CA2+ CHANNELS AND ADENYLYL CYCLASE BY CLONED µ- AND δ-OPIOID RECEPTORS IN CHO CELLS
E. A. Fitch*, P. P. Prabhu, P. Y. Law, C. J. Evans and T. G. Hales (Departments of Anesthesiology & Psychiatry, UCLA, Los Angeles, CA, 90024, and Department of Pharmacology University of Minnesota, Minneapolis, MN, 55455).

The coupling between endogenous opioid receptors and various effector systems has been studied extensively. The cloning of opioid receptor genes has facilitated research on the behavior of subtype receptor. Although there is an increasing amount of data available on the binding characteristics and coupling of these receptors to adenylyl cyclase, their interaction with Ca2+ channels has not been tested.

To investigate the coupling of µ- and δ-opioid receptors to Ca2+ channels, we have stably expressed these receptor cDNAs in GH3 cells. GH3 cells express endogenous somatostatin (SRIF) receptors, voltage-gated Ca2+ and K+ channels, but lack functional opioid receptors. Some cells transfected with the µ-opioid receptor alone (GHMOR) bound both the non-selective opioid ligand diphenorphine (Kd = 0.33 nM, Bmax = 0.39 pmol/mg protein) and the µ-selective ligand DAMGO (Kd = 1.0 nM). DAMGO dose-dependently inhibited adenylyl cyclase activity in GHMOR cells (IC50 = 21.0 nM). Using the whole-cell patch-clamp technique with Ba2+ as the charge carrier, we have found that like SRIF, DAMGO (1 μM) also inhibits voltage-activated L-type Ca2+ channels (IC50 = 104.9 nM, INH% = 26.5%). This action of DAMGO was attenuated by both naloxone and pretreatment with pertussis toxin. The selective ligand DPDEP (1 μM) inhibited Ca2+ channel activity to a much lesser extent (3.5 ± 2.1%, P < 0.05). In addition to the µ-opioid receptor, GHMOR cells were transfected with the δ-opioid receptor cDNA (GHδMOR). Expression of the δ-opioid receptor was confirmed by ligand binding studies. Like DAMGO, DPDEP also inhibited both adenylyl cyclase and Ca2+ channel activity. DPDEP (1 μM) reduced Ba2+ currents by 18 ± 9.2 ± 1.1%, P < 0.0001) in the transfected cells. We are investigating the mechanism(s) of action of µ- and δ-opioid receptors on Ca2+ channels expressed in GHMOR cells.
874.5 SPECIFIC IN VIVO BINDING OF [3H]NALTRINDEN TO DELTA OPIOID RECEPTORS IN MOUSE BRAIN. C. D. Amstett, M. Sajed, E. Akgun, and P. S. Potashne. VA Medical Center, University of Texas Southwestern Medical Center, Dallas, TX 75016.

Antinociceptive measures in vivo have demonstrated a distinction between two subclasses of delta-opioid receptors, termed delta-1 and delta-2. The delta-2 opiate receptor is selectively involved in the antinociceptive effects caused by release of endo-
genous opioids during cold water swim-stress and in the development of morphine dependence in vivo. Two subtypes of delta-opioid receptors in vivo with the ultimate goal of selectively imaging the delta-opioid receptor in the living human brain, we labeled the nonpeptide deltal-selective antagonist naltrenden (NTB) with [3H]jodo-21I by pre-incubation with our preliminary biodistribution studies of [3H]NTB in mice demonstrated a brain uptake of 1.1 percent of the injected dose per gram (53±7) at 30 min after injection with a decline to 0.84±0.45% at 60 min. [3H]NTB (1.2 μCi/g of NTB) reduced the brain retention of this radioligand by 65 percent at 60 min after injec-
tion. Pre-treatment with 1.3 μCi/g of the delta-selective antagonist bexyl-
imidazoline (BBTX) 30 min before [3H]NTB also reduced brain retention of [3H]NTB by 45 percent at 60 min after injection. At the doses used in this study, BNTX and NTB selectively inhibited antinociceptive activity of Leu- and Met-
enekaphins administered i.v. or i.r., respectively, suggesting that the expression of antinociceptive effects through interaction at deltal-opioid receptors in the brain and at delta opioid receptors in the spinal cord (Takemoto, A. E. and Porbron, P. E. Eur. J. Pharmacol., 242: 145-150, 1993).

The present results demonstrate specific binding of [3H]NTB to mouse brain deltal-opioid receptors. Inhibition of all binding pattern by pre-treatment with a pharmacologically selective dose of BNTX is an interesting finding which will require further work to elucidate the respective roles of deltal and delta2 opioid receptors.


V.A. Medical Center, Portland OR 97201 and Dept. of Pharmacology, U. of Washington, Seattle, WA 98195.

Antinociceptive compounds were used to map the presynaptic and postsynaptic locations of the kappa opioid receptor with immunocytochemistry at light and electron microscopic levels. Polyclonal, affinity purified antibodies, raised in rabbits against the unique amino acid residues 371-380 (C-terminus-KT2) and 300-312 (N-terminus-KK4), were characterized as having a high titer by ELISA and as demonstrating specific recognition of the full length kappa receptor in rat brain membranes as shown by Western blot analysis. Immunoperoxidase staining in rat tissue perfused with 4% buffered paraformaldehyde revealed kappa receptor immunoreactivity (IR) in dense patches of the dorsomedial shell of nucleus accumbens and in cell bodies and fibers of the caudate-putamen, substantia nigra, and VTA. Prominent axonal immunostaining, more effectively demonstrated by the KE4 than the KE14 antibody, was also present in the ventral pallidum, subthalamic and entopeduncular nuclei. Ultrastructural analysis revealed KT2 immunoreactivity in dendritic spines and in presynaptic terminals which made asymmetrical and symmetrical contacts with unlabeled dendrites in nucleus accumbens. The immunostaining was blocked by preabsorption with 30 μM kappa receptor peptide. This study demonstrates kappa receptor-IR in cells and fibers in the basal ganglia previously demonstrated to contain kappa receptor binding. Supported by DA 03982, DA 40412 and Dept. Veteran Affairs.

874.9 ONTOGENESIS OF δ-OPIOID RECEPTOR SUBTYPES IN RAT BRAIN AND THE STIMULATORY EFFECT OF WEANING: AUTORADIOGRAPHIC COMPARISON STUDIES. E. Knipp, F. M. Leslie, A. Borodj, O. Töhr, P. Melchiorri & L. Negri. School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK & Department of Pharmacology, University of California, Irvine, CA 92717.

We have evidence from behavioural studies that the stimulus of weaning a mother from her rat pups at day 21 activates a subtype of the δ-opioid receptor. In addition, membrane binding, western blotting and autoradiographic mapping indicate that the population of δ-receptors, primarily in the frontal-parietal cortex, recognized by [3H]-deltorphan 1 (1H-Delt) but not by the 1H-ile-1δ-leu-2 δ-1 receptor [3H-ile-2 receptor]. To determine if these receptor subtypes with different pharmacologies were associated, we carried out autoradiographic competition studies using [3H]Delt I and 2-[3H]leucine (DLT I and DLT II) on sections from weaned and non-weaned 25 day old rats. Coronal sections (25μm) were cut at the level of the caudate and adjacent sections used for determination of binding with each radioligand. Sections were cut over a total distance of 50mm for competition by DSEL and the DLT II (0.1-30nM). Slides were pre-incubated for 30 min, and binding carried out at room temperature in 50mM Tri HCl (pH 7.4) for 60 min using 1μCi of each radioligand with non-specific binding determined using naloxone (640μM). Washed and dried slides were then exposed to Hyperfilm, developed after two weeks and autoradiograms quantified by video-based computerized densitometry. Preliminary quantitation (n=8) shows that DSEL and the DLT II preferentially affect the effect of 1H-Delt I or 1H-Delt II (IC50 10-30nM) in caudate and frontal-parietal cortex. Further, there were no indications of marked differences in competition by the δ-1 ligands in weaned and non-weaned rats. The data suggest that the δ-receptors activated by weaning during development show a similar pharmacology with respect to competition by two δ-1 ligands which have been designated as subtype specific.

874.10 COCAINE-INDUCED UREGULATION OF MU OPIOID RECEPTOR MESSAGERS IN NUCLEUS ACCUMBENS IS MITIGATED BY DOPAMINERGIC MECHANISMS. B.M. Cox, A.V. Arayais, T.J. Grimm & B.J. Clock. Department of Pharmacology, Uniformed Services University of Health Sciences, Bethesda, MD 20814.

We have investigated the possibility that chronic cocaine treatment alters the levels of mRNA for mu and delta opioid receptors in brain regions rich in dopaminergic innervation. Male Sprague-Dawley rats were exposed to saline or 50 mg/kg/day of cocaine (50 mg/kg/day) for 3 days, delivered by osmotic minipump. Expression of mu and delta opioid receptor mRNA in olfactory bulb, n. accumbens and caudate putamen (caudal and rostral parts) was estimated using quantitative competitive polymerase chain reaction assays following reverse transcription. No change in the levels of mRNA for opioid receptor were detected after exposure to cocaine in any of the brain regions examined. A significant increase in the levels of mu receptor mRNA (MOR) in n. accumbens after 3 days cocaine treatment. In caudate-putamen and olfactory bulb no change in MOR mRNA was observed. In situ hybridization analysis also indicated elevated level of MOR mRNA in n. accumbens after cocaine treatment for 3 days, with little change in other brain regions. Autoradiograms of 125I-leu enkephalin, selective antagonists of dopamine D1 and D2 receptors, respectively, block this cocaine-induced upregulation of MOR in n. accumbens.

We suggest that endogenous opioid systems in n. accumbens, the brain region specifically associated with the reinforcing properties of addictive drugs, are regulated by dopaminergic mechanisms and influenced by cocaine treatment. Opioid mechanisms contribute to the development of cocaine dependence and may play a role in the development of tolerance to cocaine. (Supported by a grant from the National Institute on Drug Abuse).
SIGMA RECEPTOR REGULATION FOLLOWING CHRONIC ADMINISTRATION OF THE NOVEL SIGMA LIGAND BD1047 A.C.

The binding of radiolabeled Sigma ligands to Sigma receptors has brought to question the possible involvement of these binding sites in various movement disorders. The novel sigma ligand BD1047 has been shown to have no effect on its own, but to dose dependently attenuate dystonic postures produced by the prototypic Sigma ligands DTG and haloperidol, suggesting BD1047 acts as a sigma receptor antagonist. In order to further pursue this possibility, rats were chronically treated through the lateral ventricle with BD1047 (in artificial CSF, 10 nmol/hr) and artificial CSF alone for 7 or 14 days via osmotic minipumps. Preliminary data shows that treatment for 7 days with BD1047 results in an up-regulation of sigma receptors in whole brain (increased fmihx, no change in Kd) over regular untreated rats. Receptor levels were notably higher in the cerebellum and myelencephalon, lower in the cortex and hippocampus, and unchanged in the striatum when compared to CSF treated rats, suggesting competing/dual influences of the known antagonist vs. cytotoxic effects of BD1047 following 7 days of treatment. However, after 14 days of exposure with BD1047, there was a marked decrease in sigma receptors in whole brain, suggesting that the cytotoxic effects of the compound may predominate after prolonged administration.

CELL DIFFERENTIATION AND MIGRATION VIII

DEVELOPMENT OF OLFACTORY NEURONS AND GNIIH CONTAINING NEUROENDOCRINE CELLS IN THE ZEBRAFISH OLFACTORY ORGAN. K. L. Walzl, A. A. M. Waterfield, Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403 USA.

We are interested in mechanisms by which the olfactory placodes give rise not only to primary sensory neurons of the olfactory system, but also to neuroendocrine cells. Gonadotropin releasing hormone (GnRH) cells that migrate from the olfactory placodes to the central nervous system (CNS) are required for development of sexual maturity. Using antibodies against different forms of GnRH and other distinct populations of GnRH positive cells, we have located in different positions in the CNS. One population is present in close proximity to the olfactory organ and the post optic commissure, and the other population lies in the anterior commissure of the telencephalon. To investigate whether these cells originate in the olfactory placodes, we labeled olfactory placodes with Dil and followed cell migration in living embryos. Dil-labeled cells left the placodes and traveled to the locations where we observed GnRH positive cells. In addition, we ablated the olfactory placodes and showed that no GnRH positive cells migrate into the brain. These data suggest that the immunoreactive cells migrate from the olfactory placodes into the CNS. To learn about the origin and migration of these GnRH positive cells, we transplanted olfactory placodes from labeled donor embryos to ectopic locations in unlabeled hosts. Preliminary evidence shows that cells migrate away from the transplanted placodes. We are confirming the identity of these migrating cells and examining the details of their migration pathways.

Finally, we are beginning to investigate the lineage relationship between the olfactory placodes and the GnRH positive cells with the aim of understanding the developmental and evolutionary link between the olfactory and GnRH systems. This work was supported by MDA (KEW) and NIH HD32486 (MW).

GRANULE CELL DIFFERENTIATION IS DEPENDENT ON INTERACTIONS WITH OTHER CELLS IN THE DEVELOPING CEREBELLMUM. J. Adler1, N.K. Cho, E. Temple, and M.E. Hatten. Laboratory of Developmental Neurobiology, Rockefeller University, 1230 York Ave. NY, NY 10021.

To study the mechanism of granule cell fate specification in the developing cerebellum, we have isolated and cultured early granule cell precursors from the rhombic lip where they originate. In order to determine if isolated rhombic lip cells are competent to differentiate into granule cells, we cultured purified E14 rhombic lip cells as aggregates. Although the rhombic lip cells were dividing in vitro, they failed to extend neurites or stain with the granule cell marker TAG-1. By contrast, E17 E17, cells, which have just completed their migration from the rhombic lip over the anlage to form the EGL, but have not yet undergone massive proliferation, are already competent to extend neurites in vitro. Together these data may suggest that some interaction between the rhombic lip cells and another cell type in the embryonic cerebellum during this early wave of migration is important for rendering them competent to differentiate. To determine if these cells are competent to differentiate in vivo, we implanted E14 rhombic lip cells into E17 EGL cells into P6 EGL. Both cell populations were able to migrate and differentiate into granule cells, suggesting that the difference in their behavior in vivo reflects the different P6 environment in vivo. To determine which cell type might provide the signal which renders rhombic lip cells competent to differentiate, we have co-cultured labeled rhombic lip cells on monolayers of various cell types and assayed for neurite outgrowth. E17, P0, and E10 EGL cells can rescue neurite outgrowth of rhombic lip cells from the whole E14 anlage whereas COS5 cells cannot induce neurite outgrowth at all. Interestingly, the large cell fraction of the E17 anlage which contains glial cells, FHNJne cells, and interneurons, was also capable of rescuing rhombic lip cells. We are currently investigating the cellular source of the activity and whether the activity is a diffusible or membrane-bound factor.

GLIAL MEMBRANE PROTEINS IN THE PLASMAEMALLUM FUNCTION BETWEEN MIGRATING NEURONS AND RADIAL GLIAL CELLS REGULATE CORRECT EMIGRATION OF RADIAL GLIAL CELLS IN DEVELOPMENTAL MIGRATION. J.S. Anson1, R. Cameron and P. Rakic. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06514.

To study glial membrane proteins that contribute to the process of neuronal migration in the developing brain, we have developed a polyclonal antiserum (D4) and a monoclonal antibody (15D7) that recognize membrane proteins present at the lamellae junction between migrating neurons and their radial glial substrate (Cameron and Rakic, J. Neurosci., 14:3139). Here we tested whether these junctional membrane proteins contribute to the regulation of neuronal migration in the cerebral cortex. E17-18 coronal sections of rat cerebrum were cultured on nitrocellulose or cell-test coated coverslips. After 24 hours, the attached slices were lifted off, leaving behind 'membranes' of cerebral wall containing glial cells with migrating neurons attached to them. Changes in the migratory behavior of neurons in these implant cultures were monitored before and after the addition of purified D4 or 15D7 antibodies. The rate of neuronal migration on glial substrates was significantly reduced in the presence of these antibodies to the junctional domains. Antibody exposure often led to withdrawal of leading processes, and in some instances, to detachment of neurons from their glial substrates. Exposure to control antibodies or rabbit immunoglobulins had no detectable effect on neuronal migration. These results suggest that the glial junctional membrane proteins recognized by these antibodies are crucial for the maintenance of normal neuronal migration. Dismantling of neuron-glial junctional complexes formed by these membrane proteins may underlie neuronal detachment from glial substrates at appropriate positions in the developing cortical plate. (Supported by NS22807)

Microtubule-associated protein 2 (MAP2) is known to promote the assembly and stabilization of microtubules and hence may be important in neuronal differentiation. In this study, we describe the expression of different MAP2 mRNA isoforms and protein isoforms during the development of rat cerebellar granule cells over a 21 day period in vitro. The expression of rat cerebellar granule cells was studied by using the cell type-specific antibody. MAP2 isoforms were identified by Western blot analysis. The expression of MAP2 isoforms was found to be regulated during neuronal differentiation. The expression of MAP2 isoforms varied with the stage of differentiation and the type of neuronal cell. The results suggest that the expression of MAP2 isoforms is regulated during neuronal differentiation and the identification of the specific isoforms is important in the understanding of neuronal differentiation.

875.6 MEF2 ANTISENSE OLIGONUCLEOTIDES INHIBIT DIFFERENTIATION OF HIPPOCAMPAL NEURONS IN CULTURE. Dimas R. Davila*, Adriana F. Casimiro, Kenneth S. Kousik, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, Center for Neurologic Diseases, Brigham and Women's Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Myocyte enhancer factor 2C (MEF2C) belongs to a superfamily of transcription factors that play a role in neuron and muscle development. In the nervous system, MEF2C is expressed in excitatory neurons, and its expression is required for the differentiation of hippocampal neurons. However, the role of MEF2C in hippocampal neuron differentiation is not well understood. In this study, we investigated the effect of MEF2C on hippocampal neuron differentiation and its role in the regulation of neuronal differentiation. We found that the expression of MEF2C was regulated during neuronal differentiation and that the inhibition of MEF2C expression by antisense oligonucleotides (ODNs) reduced the expression of MEF2C in hippocampal neuron differentiation and neurite outgrowth. These results suggest that MEF2C might play a role in the regulation of neuronal differentiation and in neurite outgrowth.

875.7 THE HEAT SHOCK RESPONSE OF PC12 CELLS IS DIMINISHED UPON NEURAL DIFFERENTIATION: RELATIONSHIP TO KEY TRANSCRIPTION FACTORS. D. S. Sawrey, Y. Liu, S. Miao, and R. L. Bradley. Departments of Psychiatry and Pharmacology, LSU Medical Center- Shreveport, LA 71130.

The production of heat shock proteins (Hsps), including Hsp70, Hsp60, and Hsp90, by PC12 cells was monitored as a function of neuronal differentiation. Normal, undifferentiated PC12 cells, which are derived from the rat adrenal gland, were cultured in vitro and then exposed to heat shock. The expression of Hsps was monitored using Western blot analysis. The results showed that the expression of Hsps was diminished upon neuronal differentiation. This was due to the downregulation of key transcription factors that are involved in the production of Hsps.

875.8 NEUROTROPIC ACTIVITY OF STEM CELL FACTOR AND MELENOUSC NEUROPEPTIDE ACTIVITY OF NEUROTROPINS IN NEURAL CREST CELLS. M. Sieber-Blum* and J. Sadowski. Dept. of Cell Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Neurotrophic activity of stem cell factor (SCF) and melano-US neurotropic activity of neurotrophins in neural crest cells was studied. The results showed that SCF and melano-US neurotropic activity of neurotrophins were decreased upon neuronal differentiation. This was due to the downregulation of key transcription factors that are involved in the production of neurotrophins in neural crest cells.

875.9 DYNAMIC ANALYSIS OF TRUNK NEURAL CREST MIGRATION IN THE AVIAN EMBRYO. C. E. Knoll, A. Collazo, S. E. Fraser, M. Bronner-Fraser. 1st Dev. Biol. Ctr., UC-Irvine, Irvine, CA 92717, and Dept. of Biol., Cal. Inst. of Tech., Pasadena, CA 91125.

Trunk neural crest cells migrate through the somites in a segmental fashion, entering the neural crest but not the somitic sotoblasts. Various attractive and inhibitory molecules are thought to influence the patterning of trunk neural crest cells. It has been difficult, however, to observe the dynamic aspects of neural crest cell migration in vivo. To study the migration of trunk neural crest cells, we have developed a novel in vivo assay using a transgenic mouse line that expresses lacZ in trunk neural crest cells. The results showed that the migration of trunk neural crest cells is regulated by the expression of key transcription factors. This is important in understanding the dynamic aspects of neural crest cell migration in vivo.


We recently showed that electrophysiological coupling is quite strong among embryonic cells destined to become neurons, and that strength of coupling changes during neuronal differentiation (Dev. Biol., 147, 330-350, 1993). However, we have not determined whether the coupling is necessary or whether a decrease in coupling is an obligatory step in the process. To address this issue, we used an SV40 TS-immortalized rat hippocampal neuronal cell line and a modified electrophysiological assay. The results showed that the strength of coupling decreases during neuronal differentiation. This is important in understanding the role of electrophysiological coupling in neuronal differentiation.
MN20/D2 CYCLIN EXPRESSION REGULATES DIFFERENTIATION OF CEREBELLAR CELLS IN CULTURE CJ Mac Nab* & ME Ross, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

The D type cyclins are key regulators of the G1 to S phase transition of the cell cycle, and an integral part of cell growth and differentiation. We previously cloned from cerebellum a message form, MN20, of the D2 cyclin gene. Neural precursor cells from primary rat fetal cerebellum, MN20 has a highly restricted temporal and anatomic expression pattern in brain suggesting a role in the transition of the neuroblast from proliferation to a more mature neural morphotype. In this study, we used antisense oligodeoxynucleotides (oligos) to investigate the effect of downregulation of MN20/D2 cyclin expression on the differentiation of cerebellar granule precursor cells. Phosphorothioate derived antisense (AS)oligos complementary to sequences in the 5' end of the cDNA and their sense controls were synthesized. Cerebellar granule precursor cells were isolated from mouse on postnatal day 2,5, and plated in serum-free medium. After several hours, triplicate cultures were treated with 5, 10, or 20 μM AS-oligos as morphology of the cells was examined at 10-12 hr intervals. An AS1-oligo, targeted near the translation start codon, delayed neurite outgrowth up to 42 hr, while cultures receiving no or sense-stand oligos put out neurites by 24 hr after plating. The number of cells affected by this delay increased in a dose-dependent fashion. Many of the differentiation-delayed cells died in culture by 36 hr. Surviving cells extended poorly neurites starting around 42 hr, as determined by immunohistochemical labeling of neurofilament. The AS1-oligo effect on cell differentiation and survival was partially rescued by BFGF. The AS1-oligo effect was sequence specific since an oligo that hybridized to a region upstream of the AUS, and therefore was not expected to interfere with translation of the cyclin mRNA, had little effect on cultures. These results suggest that proper regulation of D2 cyclin expression is required for successful differentiation and survival of cerebellar granule neurons.

THE ROLE OF p35/CDK5 IN NEURODEGENERATIVE DISEASE OF THE CEREBELLUM

875.11

ACUTE NEUROTOXICITY OF Z-NH2-MNPT: CHANGES IN REGIONAL SEROTONIN, 5-HIAA, NOREPINEPHRINE, DOPAMINE AND GABA LEVELS IN SWISS WEBSTER MICE. A. M. Andrews*, D. B. Miller, J. D. O’Callaghan and D. L. Murphy, Laboratory of Clinical Science, NIH, Bethesda, MD 20892.

Our previous work has shown that Z-NH₂-MNPT induces substantial depletions in cortical and hippocampal serotonin and norepinephrine lasting as long as 6 months without compromising cell survival in striatal dopamine neurons (Andrews, A. M. and Murphy, D. L., J. Pharmacol. Exp. Ther. 287, 1432-1439, 1993). In the present study, we sought to extend our observations to include the acute effects of Z-NH₂-MNPT on regional brain neurotransmitters. Administration of 1-methyl-4-(2'-aminoethyl)-1,2,3,4-tetrahydrodipyrindine (Z-NH₂-MNPT; 4 x 20 mg/kg ip, as 2 hs intervals) to mice caused large decreases in cortical and hippocampal 5-HT and NE measured 30 min, 2, 4, 24, and 72 hrs after treatment. 5-HT was decreased by 70-90% and NE by 80-100% (p<0.01) at all times examined. Both 5-HT and NE were at or near their lowest levels within 20 min of the last injection of 2-NH₂-MNPT. In contrast, the serotonin metabolite 5-HIAA was decreased only 20% at the earliest time points in frontal cortex and hippocampus. 5-HIAA levels decreased to fall until they were depleted ~60% at 48-72 hrs after treatment. Acutely, striatal serotonin was significantly decreased by 20-40% up to 48 hrs after the last injection of 2-NH₂-MNPT but returned to control level by 72 hrs post-treatment where it remained throughout the 3 week period studied.

Neurotoxicity was determined by immunocytochemical changes in glial fibrillary acidic protein in various brain regions. GFA levels were increased by 130% of control in cortex, hippocampus, and brain stem 48-72 hrs post-treatment (p>0.05); however, the observed increases following Z-NH₂-MNPT were modest in comparison to those previously reported increases in the order of 300% of control GFA at comparable times following MPPT treatment (O’Callaghan, J. P., Miller, D. B., and Reinhard, J. J., Brain Res 521, 73-80, 1990).

786.2


Pallidal lesions characterized by increased signal intensity on T₁-weighted MR (National MR Imaging Research Centre, Montreal) images were observed in cirrhotic patients with mild to severe HE. Since one potential cause of T₁ shortening is Mn deposition in brain, Mn concentrations were measured using atomic absorption microscopy in autopsy brain tissue from 9 cirrhotic patients who died in hepatic coma and an equal number of control subjects. Mn concentrations were selectively decreased in pallidum of HE patients (control: 0.40 ± 0.04 HE patients: 5.40 ± 2.00 μg/g dry weight, p<0.01). In view of reports that chronic Mn toxicity results in alterations of the dopaminergic system that could play a role in the pathogenesis of HE, we determined the Mn signal intensity in cirrhotic patients and Mn neurotoxicity with coadministration of MPTP, an important role in the pathogenesis of drug-induced Parkinsonism. Mn levels were decreased in pallidum of HE patients (by 44%, p<0.01). Mn deposition in pallidum could explain the MRI signal hyperintensity in cirrhotic patients and Mn neurotoxicity with coadministration of MPTP. This study suggests that Mn is an important factor in human HE.

(Funded by MRC Canada).
786.3


There is evidence that metabolic defects may be a causative factor in Parkinson's disease as well as other neurodegenerative diseases. It is unclear at present how a generalized mitochondrial defect could be an underlying cause of the disease. This study was done to examine the relative vulnerability of nigral neurons to metabolic stress caused by the inhibition of succinate dehydrogenase (SDH) by 3-nitropropionic acid (3-NPA), the administration of malonate, or both. These inhibitors produce different severities of metabolic stress. These inhibitors were used in combination with 3-NPA or malonate to cause a dose-dependent loss of DA. The results suggest that global metabolic stress protects nigral neurons. The degree of protection was equidimensional to the DA population, whereas malonate was without affect. To determine the severity of metabolic stress imposed by 3-NPA or malonate, lactate production was measured in 12-day cultures treated with 3-NPA or malonate for 3 hr. Lactate production was 18% higher in 0.5mM 3-NPA treated cultures and 18% higher in 50mM malonate treated cultures when compared with untreated controls. Additionally, a 3 hr treatment with 0.5mM 3-NPA was sufficient to reduce DA uptake by 84% when measured after 48 hr of recovery. 3 hr exposure to 50mM malonate did not produce toxicity. These studies suggest that 3-NPA causes a greater inhibition of oxidative metabolism. The presence of the NMDA antagonist MK-801 (1uM) during 24 hr of 3-NPA or malonate treatment completely protected against DA and GABA loss with 50mM malonate or 0.25 mM 3-NPA and partially protected versus 0.5mM 3-NPA. These results suggest that in vitro do demonstrate a relative vulnerability to metabolic stress as compared with mesencephalic GABA neurons. Protection by MK-801 suggests that mild metabolic stress triggers a secondary excitotoxicity mediated by NMDA receptors. This work was supported by a grant from the National Parkinson Foundation.

786.4


There is evidence that metabolic defects may be a causative factor in Parkinson's disease as well as other neurodegenerative diseases. It is unclear at present how a generalized mitochondrial defect could be an underlying cause of the disease. This study was done to examine the relative vulnerability of nigral neurons to metabolic stress caused by the inhibition of succinate dehydrogenase (SDH) by 3-nitropropionic acid (3-NPA) or malonate, or both. These inhibitors produce different severities of metabolic stress. These inhibitors were used in combination with 3-NPA or malonate to cause a dose-dependent loss of DA. The results suggest that global metabolic stress protects nigral neurons. The degree of protection was equidimensional to the DA population, whereas malonate was without affect. To determine the severity of metabolic stress imposed by 3-NPA or malonate, lactate production was measured in 12-day cultures treated with 3-NPA or malonate for 3 hr. Lactate production was 18% higher in 0.5mM 3-NPA treated cultures and 18% higher in 50mM malonate treated cultures when compared with untreated controls. Additionally, a 3 hr treatment with 0.5mM 3-NPA was sufficient to reduce DA uptake by 84% when measured after 48 hr of recovery. 3 hr exposure to 50mM malonate did not produce toxicity. These studies suggest that 3-NPA causes a greater inhibition of oxidative metabolism. The presence of the NMDA antagonist MK-801 (1uM) during 24 hr of 3-NPA or malonate treatment completely protected against DA and GABA loss with 50mM malonate or 0.25 mM 3-NPA and partially protected versus 0.5mM 3-NPA. These results suggest that in vitro do demonstrate a relative vulnerability to metabolic stress as compared with mesencephalic GABA neurons. Protection by MK-801 suggests that mild metabolic stress triggers a secondary excitotoxicity mediated by NMDA receptors. This work was supported by a grant from the National Parkinson Foundation.
Cytoskeletal alterations occur in a variety of toxicant-induced neuropathologies and may play a critical role in axonal degeneration. A single exposure to compounds such as diisopropyl phosphorofluoridate (DFP) can produce OPIDN, characterized by ataxia progressing to paralysis with a central-peripheral distal axonopathy. We previously demonstrated cytotoxicity and morphological changes in neurons treated with organophosphorated neurotransmitters (PFN) and CaM kinase II (CaMKII) occur in axons early in OPIDN. The immunoreactivity for PFN and CaMKII is lost prior to the appearance of axonal various characteristics of the late stages of OPIDN. To further characterize cytoskeletal abnormalities and axonal degeneration, sections of spinal cord from control, DFP, and tissue killed 7, 10, 14 and 21 days after administration of 1.7 mg/kg DFP, were stained with a monoclonal antibody recognizing the alpha and beta subunits of tubulin. In control animals, the tubulin antibody labels the central core of axons, similar to PFN and CaMKII antibodies. The tubulin antibody also labels the cytoskeletal aggregates detachable in axons in early stages of OPIDN. But unlike PFN or CaMKII, prominent axonal various characteristics of late stages of OPIDN. These observations indicate that neurofilaments and tubulin are differentially altered in OPIDN.

878.11 EXCITOTOXIC-DEPENDENT DNA FRAGMENTATION AND FREE RADICAL FORMATION PRODUCED BY 2-METHOXYHIDAZOL-4-CARBONYLACID (2-ICA). A NOVEL HIPPOCAMPAL NEUROTOXIN. R.S. Binger, G.K. Wang and G.B. Ison. Dept. Pharmacology and Toxicology, Pennsylvania State University, College of Medicine, PA, 17707.
We previously demonstrated that 2-ICA, a major metabolite of cyanide, produced seizures in mice that were qualitatively and quantitatively similar to seizures observed with glutamate and were blocked by glutamate antagonists. Unlike known glutamate neurotoxins, 2-ICA administered i.c.v. produced lesions primarily confined to the CA1 region of the hippocampus, similar to an excitotoxic model of transient global ischemia (Binger et al., Neurotoxicology 16:115, 1995). Failure of MK-801 or CNQX to protect against 2-ICA induced lesions suggested that 2-ICA may have been differentially involved in CA1 neurotoxicity. Because excitotoxicity is normally associated with delayed necrotic cell death, the goal of this study was to determine if 2-ICA induced apoptotic changes. The results of this study show that 2-ICA (0.32-3.2 mM) increased the rate of formation of reactive oxygen species (ROS) in acutely dissociated neuronal cultures using the fluorescent probe 2,7-dichlorofluorescein. Consistent with an excitotoxic-independent action, the increased rate of formation of ROS by 2-ICA was not significantly reduced by MK-801 (1.0 mM), but was blocked by the free radical scavengers, n-acetyl-L-cysteine (100 mM) or catalase (100 U/ml). Forming agents of DNA fragmentation by 2-ICA (2.5 µmol) in mouse produced DNA fragmentation primarily in CA1 hippocampal neurons. This study shows that 2-ICA induced DNA fragmentation may be due to DNA fragmentation caused by the formation of ROS and that 2-ICA may be useful in studying the role of apoptosis in hippocampal neurodegeneration. (Supported in part by NIHES grant 84140).

ALZHEIMER'S DISEASE: MECHANISMS OF DEGENERATION II

878.7 REGIONAL SPECIFICITY OF INCREASED BASIC FIBROBLAST GROWTH FACTOR (FGF-2) IN ALZHEIMER'S DISEASE. E.J. Stapa*, M. Carlsson, M. Rodriguez-Woll, M. Yous, M. Kohn, A.M. Gonzalez and A. Brest. Brown University, Providence, RI, The Picower Institute, Manhasset, NY and The Scripps Research Institute, La Jolla, CA.
Basic fibroblast growth factor is increased in the perforant cortex and hippocampus of Alzheimer(AD) brains (Stapa et al., BrBRC 171:690-696, 1990). Both of these regions are known to be severely affected in AD. It was therefore unclear whether the FGF would be increased in the less affected areas such as the amygdala. These areas are severely involved in the disease; A4 and tau proteins are found in AD and ADAD patients. Immunodensity measurements of FGF were obtained in amygdala, A10/A20, and hippocampus, and area regions which are less severely involved by the disease; A44 and striatum, in 14 and 14AD patients. Immunodensity measurements were also performed for synaptophysin and B amyloid. The immunocytochemical procedures utilized specific antibodies raised against FGFII (Biosource, Inc.), synaptophysin (Biosource) and B amyloid (13,400 dalton, GE Laboratories). Denysmitograph measurements of mastic product were accomplished using NIH Image 1.51 and averaging data from 10 randomly selected 400 paraffin micrographs in each region area. The area measure was of FGF protein as AD positive pixels/control positive pixels indicated a robust fold increase in affected areas A10/A20, A42/A43, and 2:1.0 vs less involved regions A44 and striatum. Immunodensity measurements for synaptophysin were consistent with synaptophysin loss in all brain regions. A10: 92=92-92, A42: 94=94, s=97. Similar measurements of amyloid burden indicated an increased deposition of Beta amyloid A10=66, 1.0=1.0, A42=94, A43=94, s=12.5. These data indicate that FGF is preferentially increased in brain areas severely affected by AD and suggest that this increase may be related to multiple factors including synaptic loss and B amyloid deposition.

878.8 DIFFERENTIAL BRADYKININ-INDUCED CA2+ ELEVATION IN FIBROBLASTS FROM ALZHEIMER'S VS. CONTROL DONORS. N. Hashimi, R. Eichler, M. Barchi, S. Bergamaschi, F. Battiston, S. Grosswald and D.J. Allsopp. National Institute on Aging, Bethesda, MD 20892.
A number of studies have indicated that neural tissues of Alzheimer's Disease (AD) patients exhibit alterations at the cellular and molecular level. We have recently identified molecular AD patients including: functional absence of a n-113 p 106 TEA-sensitive K+ channel, enhanced bombesin-induced Ca2+ release, and elevated production of GTP binding protein. This study explores the bradykinin(BK)-induced Ca2+ release in a new and extended population of AD and control donors. Fibroblasts were obtained from the Coriell Cell Repository, and from the National Institute on Aging, National Institutes of Health, Bethesda, MD 20892. Cells were cultured in 96-well plates in 250 ul of 10%FCS. At 24 hr cells were treated with BK (0.125-500 nM). 120, 12 out of 14 AD cell lines had clear responses, p < 0.001 (Mean Whitney). The difference between the two groups analyzed by a contingency table (responses/no response) was also highly significant, p < 0.0001 (Fisher's exact test). Cell lines from 'escapes' showed responses similar to those of control cell lines. BK concentrations of 1 and 10 mM elicited similar responses in AD and control donors. These observations confirm and extend previous studies indicating that BK-mediated Ca2+ release is enhanced in AD fibroblasts. In addition, these results provide another parameter whereby AD patients might be diagnostically differentiated from controls.
T87.3
ALTERED PRESYNAPTIC PROTEIN NACP IS ASSOCIATED WITH PLAQUE FORMATION IN ALZHEIMER’S DISEASE. T. Salt
al*, A. Iwai, M. Mallory and E. Masliah. Dept of Neurosciences, Sch. of Med., Univ. of California-San Diego, La Jolla, CA 92039
We have recently identified, in the brain tissue of patients afflicted with AD, the NACP (non-AD component of amyloid) as another constituent of amyloid. NACP is derived from a larger precursor, NACP, a presynaptic protein. The semiquantitative immunohistochemistry demonstrated that the properties of NACP are similar to those of NACP in synaptosomes, suggesting a role in synaptic plasticity. However, the formation of C-terminal fragments of NACP was not increased in the AD frontal cortex as compared to controls. NACP was localized to approximately 80% of the synaptosomes-immunoreactive boutons, presumably the presynaptic terminals, and to the dystrophic neuocentric component of the plaques. Although the overall numbers of NACP-positive boutons were reduced, there was significant increase in the intensity of NACP-immunoreactivity per bouton in AD. This increased intensity of immunoreactivity of boutons in AD was not observed with anti-synaptophysin, consistent with immunohistochemical labeling of antibody.NACP immunoreacted with amyloid in 35% of the diffuse plaques and 55% of the mature plaques. Normal aged brains contain small groups of disk-like plaques were negative with anti-NACP. Double-immunostaining with Aβ antibodies showed that NACP immunoreactivity is more abundant in the center portion of amyloid plaques than in the periphery. These studies suggest that there is a connection between metabolism of presynaptic proteins and amyloid generation, and that NACP might be involved in the formation of compact amyloid and mature plaques.

T87.5
INDUCTION OF HEME OXYGENASE-1 mRNA IN CEREBRAL VESSELS AND NEOCORTEX IN ALZHEIMER’S DISEASE. D.B. Premkumar*, M.A. Smith, B.K. Kottu, B. Wippert, G. Perry and R.N. Berliner. Department of Neurological Surgery and Case Western Reserve University, Cleveland, Ohio and The National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA
Previous studies demonstrated the specific association of heme oxygenase-1 protein to the pathological lesions present in Alzheimer’s disease (AD). In this study, we used reverse transcription-polymerase chain reaction methods to show the increased expression of HO-1 but not hemoglobin-2 HO-2 mRNA transcripts in cerebral neocortical and neostriatal vessels from subjects with AD compared to age-matched non-AD controls. Neither HO-1 nor HO-2 mRNA was altered in the cerebellar samples; a brain region usually spared from the pathological alterations of AD. There was no clear evidence that the expression of HO-1 in these tissues was related to postmortem interval, the shown postmortem agonal factors. The specificity of our observations was further verified by the demonstration of unchanged β actin mRNA transcripts but increased GAP-45 mRNA in tissues from AD subjects compared to controls. Our findings indicate the specific induction of HO-1 mRNA and increased expression of HO-1 protein in neocortex and cerebral vessels but not HO-2, and suggest that oxidative stress is important in the pathogenesis of AD. Supported by grants from NIA and ARDRA.

T87.6
We applied a dual channel NIRS system using two pairs of optodes placed on the left parietal cortex and on the right parietal to study non-invasively changes in cerebral hemoglobin oxygenation during activation of brain function in patients with Alzheimer’s disease (AD) compared to controls. Healthy elderly subjects (n=7; age 60 ± 15) showed increases in oxygenated hemoglobin [HbO2] as well as total hemoglobin [HbT] in both frontal (mean (arbitrary units) ± SEM, 2.00 ± 1.45 and 2.37 ± 1.39, respectively) and parietal cortex (1.26 ± 1.03 and 0.23 ± 0.88, respectively) during performance of a verbal fluency task. In contrast, AD patients (n=10; age 65 ± 13 years) showed decreases in [HbO2] and [HbT] in the parietal cortex (-2.48 ± 0.91 ± SEM, p<0.002, and -3.37 ± 1.13 ± SEM, p<0.05, respectively) and, simultaneously, increases in [HbT] and [HbO2] in the frontal cortex (1.40 ± 2.56 and 0.31 ± 0.30, respectively). Simultaneous rCBF and [HbO2] PET measurements during brain activation (strokeweight, n=10) showed that rCBF changes were parallelised by changes in [HbO2] as measured by NIRS. Modeling the spatial relationship of the NIRS-measurements to the PET-image revealed best correlation assuming a NIRS sample volume covering approximately the outer 1.6 cm of the brain cortex beneath the optodes (r=0.71, p=0.021). Regional decreases in cerebral [HbT] and rCBF during activation of brain function in patients with AD may contribute to the development and the course of neurodegeneration.

T87.8
POSTISCHEMIC DEPLETION OF GAP REACTIVITY IN HIPPOCAMPAL ASTROCYTES IS PREVENTED BY HWA285. A. McRae*, M. Knobel, P. Schubl, R. Radulovitch.
1University of Göteborg Sweden 41390, 2Max Planck Institute of Psychiatry Martinsried Germany, 3Hoechst AG Frankfurt a.M. Germany
Transient (5 min) forebrain ischemia has been shown to reduce the reactivity of astrocytes in the gerbil hippocampal CA1 area, reflected by an increased immunoreactivity for glial fibrillary acidic proteins (GFAP) at the 2nd postischemic (p.i.) day. In animals developing a complete cell loss of CA1 neurons in the ischemic cell body area, this is followed by a depletion of GFAP staining in the CA1 stratum radiatum which appears at the 7th p.i. day as a demarcated area with a complete loss of cellular and astrocytic GFAP staining. Previous studies have observed that pre-treatment with the neuropeptidergic pharmacophore propentofylline (HWA285) interferes with the initiation of the astrocyte reaction preventing the ischemia-induced increase in GFAP staining. However, daily post-treatment with HWA285, started at the 2nd p.i. day was found in the present study to prevent the consecutive fading of the GFAP reactivity completely at the 13th p.i. day in the CA1 area. Here, a maintained strong GFAP staining was observed in process-bearing astrocytes, whereas neuronal damage was not prevented (as verified by MAP2 staining). We have employed a similar ischemic model in the rat which demonstrates that increasing GFAP reactivity in cultured astrocytes and reinforces the development of potentially neuroprotective properties by strengthening the cAMP signaling, whereas GFAP immunoreactivity is suppressed. Such a pharmacological conditioning of cells may provide protection against progressive, less dramatic neuronal death than induced by ischemia, e.g. in Alzheimer or Parkinson patients.

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787.9
ALZHEIMER'S DISEASE: MECHANISMS OF DEGENERATION II

787.10
CHRONIC EXPRESSION OF PLATELET ACTIVATING FACTOR (PAF) AND CLUSTERNER IS ASSOCIATED WITH NEURODEGENERATION IN RAT BRAIN FOLLOWING EPILEPTIFORM SEIZURES. Benzel, S.A.1,2; Benzel, E.M.P.R.1,2; Brown, B.1,2; 1,2Tenn.

Pro-inflammatory molecules are associated with the principal lesions of a wide variety of progressive neurodegenerative disorders. It is unclear whether induction of inflammatory response molecules proceeds preceding lesion formation or is a consequence of chronic exposure to non-degradable plaque elements (i.e., Periodic acid Schiff (PAS) positive elements). In the present study, we investigated the relationship between clusterner, PAF, and the development of PAS+ lesions following laminar status epilepticus. Clusterner is a complement regulatory protein and a prominent marker of neurodegenerative and apoptotic, PAF is a potent pro-inflammatory mediator implicated in mediating hemorrhage and convulsive activity. In the present experiments, epileptiform seizures were elicited in male Wistar rats by intraperitoneal injection of 50 mg/kg of pentylenetetrazol. Brains were harvested at various time points after seizure induction for PAS histochimistry and clusterner, PAF, and PAF receptor (PAFR) expression by immunohistochemistry and in situ hybridization. Aprotinin was determined in vivo TUNEL labelling and by evaluating DNA fragmentation. RNA was extracted from unfixed tissue blocks and regional changes in PAF and clusterner mRNA expression were evaluated by Northern blot and RT-PCR. Results demonstrate that epileptiform seizures induce both programmed cell death and PAS staining 24-168 hr after seizure onset. In significant interest was the finding that changes in PAF expression and PAF expression were correlated with significant alterations in clusterner expression, PAS staining, and apoptosis. These changes were also observed in a variety of brain areas that demonstrated only limited cell loss. Chronic PAF and clusterner expression occurred in these areas expressing clusterner at later times exhibiting substantial cell loss and PAS+ lesions. These data suggest that chronic exposure to pro-inflammatory agents precedes and may, in fact, contribute to the etiology of neurodegenerative lesions. (Supported by M.R.C.)

787.11
ANTI-MICROGLIA ANTIBODIES IN SERA OF ALZHEIMER'S PATIENTS. H.S. Lemke*, N. Graf*, A. Hennemann*, Dept. Psychiatry, Medical Center, D-85049 Ingolstadt, Germany (M.R.L.), Dept. Neurology, Ulm University, D-89070 Ulm, Germany (M.G.A.H.)

Objective: Immune mechanisms seem to be involved in tissue destruction in Alzheimer's disease (AD). There is evidence indicating the importance of microglial response in AD. The present study was undertaken to study if patients with AD show a response to anti-microglial antibodies against various brain structures.

Methods: A cohort of 24 patients was studied with AD (according to DSM-III-R and NINCDS-ADRDA criteria, MMSE=16) and matched controls (n=30, MMSE=24) serum antibodies were measured using an indirect immunofluorescence test as published previously.

Results: In nine perinuclear antibodies against microglia were found in amygda and frontal cortex. One only of the controls showed antibody binding to microglia.

Conclusions: The results support the hypothesis of involvement of immune mechanisms in AD. Perinuclear anti-microglia may play a role in tissue destruction of AD. Whether these findings are specific for AD or represent a subgroup of AD needs further evaluation.

787.12
EFFECT OF ATP DEPLETION ON THE ACTIVITY OF PKC\textsuperscript{\textregistered} IN DIFFERENTIATED PC12 CELLS. IMPLICATION FOR ALZHEIMER'S DISEASE. I. Ye, M.K. Metcalfe, L. Butman*, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Hyphosphorylated Tau is a major constituent of tangles found in Alzheimer brains, ATP is depleted in such brains. We previously reported that the brain protein kinase PKC\textsuperscript{\textregistered} can hyphosphorylate Tau to resemble Alzheimer Tau, as found in the tangles, it is strongly inhibited by free ATP in excess over MgATP. The mitochondrial uncoupler FCCP will deplete ATP levels in differentiated PC12 cells. (1) While in-gel kinase assays show that FCCP treatment inhibits the activities of 4 MBP protein kinases by an unknown mechanism, the same assay shows the activity of another kinase to be unaffected. This appears to be PKC\textsuperscript{\textregistered} as by direct assay, by indirect assay, and by transient transfection with hTau40 or neurofilament proteins as substrates. The increased in-vivo activity of a neurofilament protein reported by us [Bush et al.,PNAS 1995, 92:1617] is therefore likely to be due to up-regulation of the kinase in the cell by ATP depletion [release from partial inhibition]. The PKC\textsuperscript{\textregistered} assay of ERK2-specific immune-precipitates from extracts of treated cells also shows no in-vitro increase of kinase activity. FCCP does not increase synthesis or tyrosine phosphorylation of PKC\textsuperscript{\textregistered}.

(2) FCCP treatment increases, mechanism unknown, the amount of protein phosphatase PP2A in extracts. This may account for the increase in the dephosphorylated Tau-1 epitope, reported by us earlier. The results explain the lack of Tau+ hyperphosphorylation in Alzheimer disease and suggest a novel mechanism for the regulation of certain kinases.

LONG-TERM POTENTIATION: PHYSIOLOGY VII

788.1
ACTIVATION OF POSTSYNAPTICALLY SILENT SYNAPSES DURING PAIRING-INDUCED LTP. D. Liao* & R. Malinow, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY and *Dept. of Physiology & Biophysics, U. of Iowa, Iowa City, IA.

We have tested the hypothesis that in the CA1/subiculum region of hippocampus there exist synapses with only NMDA receptors (and thus effectively silent at resting potentials), and these synapses could be made completely functional by pairing stimuli. According to this hypothesis, more synaptic failures were observed at hyperpolarizing (-65mV) than at depolarizing (+55mV) holding potentials (n=25, 52% vs 8% 24-72 hrs). The difference in failure rates at -65mV and +55mV was highly significant at p<0.001 (n=12). To stimulate just pure NMDA synapses, a (sub)minimal stimulus can be set so that all trials produce failures at -65mV, but responses are seen at +55mV. We recorded 100 consecutive trials at -65mV with a subminimal stimulus. Pausing the LTP protocol after the same subminimal stimulus produced subsequent synaptic responses at -65mV (12 of 22 experiments compared to 3 of 17 with no pairing; p < 0.05, Chi-test). In other experiments we could identify failures at hyperpolarizing and depolarizing potentials both before and after LTP induction. We observed no significant decrease in failure rate at hyperpolarized potentials, but no significant change at depolarized potentials (56% vs 35-46% at -65mV vs 19-4% to 22-5% at +55mV, n=12).

Synaptic specificity of LTP in CA1 pyramidal neurons has been attributed to compartmentalization of calcium in the spine correlated with the activated synapses. This hypothesis was examined by testing for synaptic specificity at excitatory synapses in the CA1 pyramidal cell layer which are largely aspino1. A standard hippocampal slice preparation from young rats (17-24 days) was used, maintained at 36 °C, and bicuculline (10μM) was added to the ACSF. Intracellular recordings from interneurons were distinguished from recordings from pyramidal neurons by their short spike duration (i.e., 4 ms), pronounced spike afterhyperpolarization and lack of spike adaptation. Two types of current step were evoked from two stimulation sites in stratum radiatum: one near the cell body layer and the other adjacent to the stratum pyramidale. Stimulus strengths were adjusted to evoke EPSPs in the range 1-5 mV. EPSPs were evoked from each input at 0.5 Hz to obtain baseline responses. Independence of the two inputs was demonstrated by lack of occlusion when both inputs were stimulated simultaneously. One input was then tetanized at 100 Hz in five bursts of 20-40 stimuli while the stimulus was abolished from the other input. During conditioning the cell was depolarized until it discharged at a high frequency. Conditioned responses for both inputs were then recorded at 0.5 Hz for at least 30 min. When one input was conditioned (n=21), the non-conditioned EPSP potentiated (10), depressed (7) or did not change (4). The conditioned EPSP potentiated (6), depressed (10) or did not change (5). The frequency of these various outcomes was independent of whether the conditioned input was proximal or distal to the soma. The heterosynaptic LTP observed in these interneurons supports the hypothesis that spines confer synaptic specificity to LTP in pyramidal cells.


T88.5 SELECTIVE RECRUITMENT OF AMPA RECEPTOR MEDIATED CURRENT DURING LTP IN MEDIAL PERFORANT PATHWAY OF RAT DENTATE GYRUS. S. Wagner and J.M. Wolters, Wadsworth Center of Physiology, University of Toronto, Toronto, Canada, MSS 1A8

Whether long-term potentiation (LTP) is due to enhanced AMPA receptor mediated current alone or due to the enhancement of both AMPA and NMDA currents is controversial. In this study, we examined the medial perforant pathway synaptic transmission during LTP to address this issue. Whole-cell voltage clamp recordings were made in dentate gyrus neurons in a standard hippocampal slice preparation. Synaptic currents were evoked by stimulating the medial perforant pathway in the middle third of the dentate molecular layer. Membrane potential was held at -65 to -75 mV. Double exponential curves fitted to the decay phase of the responses revealed a large fast component and a small slow component. Using pharmacological agents we demonstrated that the small component, constituting 14 ± 7% (n = 8) of the total response, represents the NMDA current. LTP was induced by tetanic trains of stimuli (at 10 Hz, 5 trains) in the presence of post synaptic depolarization to -20 mV. The success rate of inducing LTP in individual granule neurons was only 42% but the average magnitude was large. In a representative series of nine experiments the average potentiation was 339 ± 255%. During LTP expression, only the AMPA current increased while the NMDA current was unchanged. Furthermore, the variable magnitude of LTP was directly related to the relative size of the NMDA component prior to induction of LTP. (Supported by MRC of Canada)

T88.6 THE EXTENT OF NMDA RECEPTOR ACTIVATION DURING A TETANUS DETERMINES WHETHER LTP IS EXPRESSED BY AMPA OR NMDA RECEPTORS.

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Analysis whether LTP is expressed by AMPA or by AMPA and NMDA receptors is a useful approach to determine if LTP is pre- or postsynaptically mediated, but has given contradictory results. We have now tested the hypothesis that if the AMPA-NTA of different LTP procedures would induce AMPA-NTA from that required to trigger LTP expression by NMDA-NTA. Extracellular records were made in the CA1 hippocampal region in 0.3 mM Mg2+. The NMDA component of the field EPSP (fEPSP) was isolated from the AMPA component by CNQX before and after the tetanic procedure. The three tetanic stimulations were applied (after the washout of CNQX) at weak intensity, subthreshold for eliciting EPSPs (Tw), or at strong intensity which elicited fEPSP of 0.3 mV (Tw). Tw induced LTP of fEPSPs (59 ± 8%, n=9) but not of fEPSPs (11 ± 2%). In contrast, T(w) failed to generate LTP of fEPSPs (9 ± 3%, n=9) but produced LTP of fEPSPs (89 ± 17%). In the 4 remaining cases LTP of both components were observed. In presence of the redox reagent DTNB (200 μM) or 7-Cl-Kyn (6 μM) which reduce fEPSPs by 50%, Tw produced LTP of fEPSPs (63 ± 8%, n=6) but not of fEPSPs (12 ± 4%). Under the same conditions, Tw generated LTP of fEPSPs of smaller amplitude than in control (30 ± 6%, n=8). We conclude that i) stronger activation of NMDA receptors during a tetanus train is required for inducing LTP of fEPSPs than for fEPSPs, ii) there is a bell-shaped relationship between the extent of NMDA receptor activation during the train and the magnitude of LTP of fEPSPs. Therefore, these findings reinforce the hypothesis that expression of LTP is a postsynaptic process.

T88.7 NMDA-MEDIATED ACTIVITY-DEPENDENT SHORT-TERM PLASTICITY OF ELECTRONEUTRICAL COUPING. Alberto Pereda and Donald S. Faber. Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA.

As reported previously disynaptic tetanic stimulation of the posterior eighth nerve produces a long-term homeostatic enhancement of both the electrical and chemical components of the mixed excitatory postsynaptic potential (EPSP) recorded from the Mu2nMd cell in the salamander. We report here that the same tetanizing paradigm can evoke a shorter lasting potentiation (2-10 min), which averaged 231 ± 41% (± SEM) for the electrical component and 155 ± 20% for the chemical (EPSCs) (n=14) when measured right after tetanic stimulation. These modifications were non-specific, since the Mu cell anterograde spike, an index of the neuron's input resistance, did not change significantly (mean=100±1% ; n=14). The fact that short-term potentiation of electrochemical coupling occurs in parallel with that of the chemical EPSP suggested that these transient changes might reflect a presynaptic origin, i.e., enhanced or decreased activity-dependent increase in the presynaptic calcium concentration. To test this possibility we compared the observed effects with those evoked by paired presynaptic stimuli. However, paired pulses failed to produce long-term changes in the electrical component (mean=100±2% ; n=16). In contrast, the induction of short-term potentiation still depended upon the disinhibitory or burst-like property of the underlying stimulus, which optimizes NMDA receptor activation while continuous stimulation (50 Hz) favors glutamate transmission. A short-term potentiation of short duration required NMDA receptor activation, as the intermittent stimulus bursts failed to induce any potentiation in the presence of the NMDA receptor blockers APV and CPP (30-100 μM, n=5). These potentiations were also blocked by intradendritic injections of the calcium chelator BAPTA (5mM, n=2). Thus, while paradigms known to raise the presynaptic calcium concentration were unable to evoke tetanic changes not only in the chemically mediated EPSP but also in electrochemical coupling.

T88.8 PRESYNAPTIC LONG-TERM POTENTIATION (LTP) OF EXCITATORY SYNAPTIC TRANSMISSION IN SINGLE GRANULE CELLS (GCS). G. Tong*, V.C. Malafo, G.E. Lanyi and P.A. Maglott. Dept. of Cellular and Molecular Pharmacology, Physiology and Psychiatry, University of California, San Diego, SRA143.

Previous studies in slices suggest that mossy fiber LTP in the hippocampal CA3 region is induced and expressed presynaptically by increased CAM- dependent protein kinase (PKC) application in presynaptic terminals. Moreover, we have advantage of microcultures to test whether single guinea pig GCs which form autapses show LTP. GCs were distinguished from other cells by their L-AP4 sensitivity. Consistently, the results from the slices obtained during tetanic stimulation (50 Hz, 14 times) in the presence of the NMDA receptor blockers D-AP5 (50 μM) and Mg2+ (1 mM) induced a sustained potentiation of both NMDA and AMPA components of evoked chemical currents (EPSCs) that lasted at least 30 min in L-AP4 sensitive cells. In contrast, in L-AP4 insensitive cells tetanic induction induced no LTP. Short applications (2 min) of forskolin (30 μM) also induced a long-lasting potentiation of evoked NMDA EPSCs in L-AP4 sensitive cells but a short-term potentiation (about 10 min) in L-AP4 insensitive cells. Furthermore, presynaptically elicited nonsynaptic neurons with a pKA inhibitor, BAPT-C-PAPC (100 μM) blocked tetanized induced LTP. Finally, the frequency of spontaneous miniature AMPA EPSCs was potentiated by tetanic stimulation but the amplitude of minis remained the same. These data suggest that tetracanic stimulation causes a presynaptic increase in cAMP leading to a long-lasting enhancement of transmitter release. This enhancement may be due to an alteration of the internal machinery that controls transmitter release.
788.9
PAIRED-PAUSE FACILITATION OF DUAL COMPONENT EPSCS IN HIPPOCAMPAL PYRAMIDAL CELLS - IMPLICATIONS FOR LTP.

SPINOCORDAL BRANCHES FROM CORDIAL CELLS WHICH ARE INVOLVED IN THE EMBRYONIC MOVEMENT OF SPINAL CORD AFFERENTS. THEORETICAL AND EXPERIMENTAL MODELS. A. Torres, G. Ron, and J. L. Torrelo. Department of Anatomy & Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.

In an effort to understand how lumbar and sacral spinal cord circuitry controlling function of the urinary bladder develop during the prenatal period, a retrograde fluorescence labeling technique was used to identify and describe 1) autonomic and somatic motor neurons that provide innervation to bladder wall and sphincter musculature, and 2) the growth of vesicoureteral reflex (VUR) from the bladder to the spinal cord. Crystals of the intensely fluorescent, lipid soluble tracer DiI were placed in the urinary bladder of aleatory-fixed embryos that had been taken at different developmental stages and then Askernized in vitro to fix the bladder. The bladder was attached to the spinal cord, either at the lumbar level (L5-6) by the spinal cord immediately after the retrograde fluorescence labeling technique. Results showed that the spinal cord was transected at 3 gastric, 5) lower, and 6) upper levels.

788.10
TOXIC AND PHASE RETROGRADE SIGNALING AT CA3-CA1 SYNAPSES. A. Ajofobi and K. Kullmann. Dept. of Neuroscience, S. Raffaelli, Delft, Milano, Italy.

Mechanisms of diffuse retrograde signaling at CA3-CA1 synapses and their importance for control and potentiated transmission are strongly debated. One of the main arguments against this hypothesis is that LTP cannot be induced if the postsynaptic neuron is loaded with NMDA receptors, as minimal ionotropic receptors should deliver diffusive signals. We investigated this problem in CA3-hippocampal cultures by monitoring miniature EPSCs (mEPSCs) (0.5 µM TTX present). Minimal frequency LTP was found even with NMDA (100 µM) in the bath, but in addition we could also induce bidirectional BAPTA effects. We used a dual electrode recording approach to study the influence of postsynaptic BAPTA or NO blockers on basal synaptic activity. We monitored with first pipette cells (PC) and second with an extracellular electrode and found that synaptic transmission could be induced by this technique. We conclude that the synaptic terminal functioning could be modulated by different toxic and phase retrograde signaling mechanisms controlled by postsynaptic Ca2+.

788.11
ACTION POTENTIALS PROPAGATING BACK INTO DENDRITES TRIGGERS CHANGES IN EFFICACY OF SINGLE-AXON SYNAPSES BETWEEN LAYER V PYRAMIDAL NEURONS. Henry Markram* and Bert Sakmann. Max-Planck Institute, Dept. of Cell Biology, Jena, Germany.

Somatic depolarization of single-axon (sax) synapses was examined using whole-cell recordings of synaptically coupled pairs of Layer V pyramidal neurons in the rat neocortex. Synapses were identified in slices with infrared differential interference contrast microscopy. Simultaneous pre and postsynaptic depolarizations, either in a burst action potentials (APs), triggered a long-lasting (≥ 40 min) increase in the amplitude of saEPSPs (n= 9). APs triggered in synapses in the pre and postsynaptic neurons in six (5%) neuronal pairs and resulted in a long-lasting increase in synaptic efficacy (≥ 37%) that was dependent upon the activation of NMDA receptors. These synaptic potentials in APs in the absence of synaptic input had no effect on saEPSP (n= 4). Associative subthreshold depolarization (n= 8) or depolarization to around -20 mV in QX-314- loaded neurons (n= 6) during synaptic input, also failed to induce an increase in synaptic efficacy. Indeed, strong associative depolarisation resulted in a reduction in the amplitude of saEPSPs. The maximum increase in synaptic efficacy increased with the frequency of synaptic prepotentials. While increasing the number of APs in a burst of 20 Hz did not result in larger increases than the same number of APs in the absence of prepotentials, the APs with the backgrounding AP increased in synaptic efficacy while saEPSPs that arrived 10 ms after the backgrounding AP were either unchanged or weakened (n= 8). These results show that the backgrounding AP is the trigger for changes in efficacy of this synapse. The constraints of AP frequency, number and timing suggest that connectivity between pyramidal neurons that obtain common synaptic input will be enhanced which may enable the activation of functional neuronal ensembles.

788.12
LTP INDUCED BY NIT-5 PHOTOLYSIS IN RAT CA1 HIPPOCAMPAL NEURONS WITHOUT PRESYNAPTIC ACTIVITY. D. Neveu,* R. C. Malenka,* and R. S. Zucker. *Neurobiology Div., Univ. of Calif, Berkeley, CA 94720 and 'Psychiatry Dept., Univ. of Calif, San Francisco, CA 94143.

A rise in postsynaptic Ca2+ can trigger a long-lasting form of synaptic enhancement resembling LTP in rat hippocampal CA1 pyramidal cells (Malenka et al., 1988). It is unclear whether this process is the same as the putative NMDA receptor-dependent LTP induced by strong synaptic stimulation. Neither its interaction with tetanically induced LTP nor its requirement for presynaptic activity have been determined. We used photo-activation of postsynaptically injected NMDA to elevate postsynaptic Ca2+ to a level sufficient to cause a few micromolar. This induced an increase in the rising slope of the EPSP obtained on stimulating Shaffer collateral afferents at 0.1 Hz. An increase of up to 140% was observed (average 66% ± 35, n = 14), and remained above baseline for the duration of long recordings (≥ 40 min). Similar effects were obtained when several stimulus amplitudes were used for 5 min before and after the photo-stimulating, indicating that presynaptic activity was not required for induction of this process.

When tetanically induced LTP (2-3 sets of 100-150 Hz trains for 1-2 s separated by 20 s) of a test pathway preceded the photo-stimulating 25-35 min, the photo-stimulus never increased the test EPSP; control pathways were potentiated. Omission of photo-activated LTP by tetanic LTP suggests that they share common processes of induction and/or expression.

These results also suggest that at least a moderate level of LTP can be induced without involvement of presynaptic activity. This result has implications for models of LTP that require the concurrent action of a retrograde messenger with presynaptic activity. Supported by NIH Grant NS 15114.

789.1
DEVELOPMENT OF PRIMARY AFFERENT COLONIC BLANCHES IN THE EMBRYONIC MOUSE SPINAL CORD. S. Oskar, A. B., W. D. Smiley. Department of Neurology, CSN51, Washington University School of Medicine, St. Louis, MO 63110.

The extension of colonic branches from growing axons to their target fields is a key event in the development of neural circuits. A favorable system for analysis is the colonic branching of primary afferent in the spinal cord which occurs early in the development stage. Under the influence of this study, we characterized the development of primary afferent colonic branches in the mouse spinal cord, which will provide a framework for future work on the function of transgenic animals. Primary afferent projections were visualized using Dil crystals placed in the mammary gland or thoracic nerves of mouse embryos aged between embryonic day 10.5 and 15.5. The first primary afferent axons reach the spinal cord at E10.5 and grow rostrally in the mesencephalic region of the dorsal horn. At this stage axons are relatively smooth and growth cones are prominent. There is a delay of at least 42 hours prior to the elaboration of axon collaterals branches into the spinal cord. Between E12.5 and E13.5 primary afferents develop nodes of frequent intervals, and collaterals enter the gray matter by branching from parent axons at the site of these nodes. There were many more nodes than colonic branches at all developmental stages. Nodes persisted until at least P0. The colonic branches often develop at the anterior and ventral regions of the spinal cord horn and extend their dendrites in a medio-lateral plane and into the dorsal and ventral funiculi. Primary afferents begin to enter the spinal gray matter at E15, and can be divided into two groups based on their morphology, 1) larger diameter fibers that enter the spinal cord more medially and descend to somatic motor neuron pools, and 2) smaller diameter fibers that enter the spinal cord more laterally and ramify in laminae within superficial (I and II) and deep (V-VI) laminae of the dorsal horn. The spatially- and time-dependent organization of the development of these neural elements suggests, that, as in other systems, the development of spinal cord circuits may be regulated by the expression of centrally- or peripherally-derived trophic molecules.

789.2
PRENATAL DEVELOPMENT OF SPINAL CORD CIRCUITRY INVOLVED IN URINARY BLADDER FUNCTION IN THE RAT. K. G. Suri and R. J. Todres. Department of Anatomy & Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.

In an effort to understand how lumbar and sacral spinal cord circuitry controlling function of the urinary bladder develop during the prenatal period, a retrograde fluorescent labeling technique was used to identify and describe 1) autonomic and somatic motor neurons that provide innervation to bladder wall and sphincter musculature, and 2) the growth of vesicoureteral reflex (VUR) from the bladder to the spinal cord. Crystals of the intensely fluorescent, lipid soluble tracer DiI were placed in the urinary bladders of aleatory-fixed embryos that had been taken at different developmental stages and then Askernized in vitro to fix the bladder. The bladder was attached to the spinal cord, either at the lumbar level (L5-6) by the spinal cord immediately after the retrograde fluorescence labeling technique. Results showed that the spinal cord was transected at 3 gastric, 5) lower, and 6) upper levels.

789.8
LONG-TERM POTENTIATION: PHYSIOLOGY VII

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995

To investigate factors involved in the development of limbic and nonlimbic cortical circuitry, we examined the timing of thalamo-cortical and cortico-thalamic growth in the somatosensory (Sx) and piriform (Pfh) cortices. Lophicophoric dye tracers, DlbIC18-(3) or DaA were inserted into 4% paraformaldehyde fixed brains of rat fetuses aged embryonic day (E) 13-20. Seventy-eight embryos were used. The lophicophoric dyes were inserted into either presumptive Pfh or Sx cortices, or the thalamus in single or double-labelling paradigms. The sections were examined for location of fibers extending from the insertion site and the presence and location of retrogradely labeled neurons. Results show that Pfh cortico-thalamic axons meet the thalamo-cortical axons initially in the internal capsule on day E13.5 and probably represent the first axons from the Pfh. Cortical axons arrive in the thalamus on E14-E14.5, a few days prior to axons from Sx cortex on E18 from neurons located in lateral thalamus arrive in Pfh cortex on E15 and in Sx cortex on E18, axons from neurons in midline thalamic region arrive in Pfh on E18. Neurons labeled from either cortical site formed discrete clusters in thalamic areas. Axons segregated in the lateral portion of the internal capsule, with the Sx cortical-thalamic axons more dorsally situated than the Pfh cortico-thalamic axons. These results show that while temporally distinct, limbic and nonlimbic cortical projections are initiated in similar, highly specific patterns. Supported by Temple University (M.B.) and NIMH grant 54507 to P.L.


Mouse N16K mRNA is reproducibly differentiated into neurons upon treatment with dibutyryl cyclic AMP (dbcAMP). In order to understand the molecular mechanism and cellular events which underlie neuronal differentiation, we constructed a subtractive cDNA library made from 48h-treated N202 cells. We then screened randomly selected colonies by differential hybridization using labeled cDNA probes synthesized from the dbcAMP (+) and dbcAMP (-) mRNA pools. One clone, N16K, was markedly increased in N202 cells after dbcAMP treatment for 48h. Expression of N16K mRNA in adult mouse was detected only in brain and spinal cord, but not in other non-nervous tissues. N16K encodes a protein composed of 212 amino acids. Neither the nucleotide sequence of N16K nor the deduced amino acid sequence showed appreciable homologies to known sequences in the database. Further characterization of N16K should provide additional information about its function during neuronal differentiation. (Supported by the Ministry of Education, Science and Culture of Japan and by Uehara Memorial Foundation.)

DISTRIBUTION OF TWO PRIMARY PROTEIN KINASE C (PKC) SUBSTRATES, MYRISTOYLATED ALANINE-RICH KINASE SUBSTANCE (MARKS) AND F1G4-43 mRNA IN THE ADULT RAT BRAIN. R. K. McNamara*, D. G. Watson*, R. D. Lenox*.1,2 Departments of Psychiatry1, Pharmacology2, and Neuroscience3, University of Florida College of Medicine, Gainesville, FL, 32610-2516.

MARKS (80-KDa) and F1G4-43 (85-kD noneomologus) are major substrates for PKC and involved in neurite outgrowth and development as well as in the adult brain, where F1G4-43 mRNA levels remain elevated in regions associated with the regulation of plasticity (Meberg & Routtenberg, 1991). The constitutive distribution of MARKS mRNA has not yet been documented in brain. To determine the relative distribution of MARKS and F1G4-43 mRNAs in the adult rat brain, brains were taken from Sprague-Dawley rats (250-300g) and were later perfused for in situ hybridization histochemistry. Serial sections were hybridized with radiolabeled antisense riboprobes synthesizing the nucleotide sequence of the MARKS cDNA (Seykora et al., 1991) or 779-1255 of the rat F1G4-43 cDNA (Rosenthal et al., 1987). Overall, MARKS hybridization was expressed in the majority of regions but was most pronounced in pyriform cortex, medial habenular nucleus, paraventricular hypothalamic nucleus, and the cerebellar granule cell layer. Consistent with earlier reports, prominent F1G4-43 expression was observed in several regions including thalamic and hypothalamic nuclei, lateral habenular nucleus, entorhinal cortex, raphe nucleus, and cerebellar granule cells. Within the hippocampal formation, MARKS hybridization was highest in the CA3 and dentate gyrus regions. In pyriform cortex, expression was highest in CA3 and hilar neurons, moderate in CA1 pyramidal neurons, and low-absent in dentate gyrus granule cells. While MARKS and F1G4-43 mRNAs are coexpressed in certain regions (pyriform cortex/cerebellar granule cells), their differential expression in other regions may reflect high levels of constitutive PKC activity, which has been shown to up-regulate F1G4-43 mRNA (Perrone-Bizzozero et al., 1993) and down-regulate MARKS mRNA (Brooks et al., 1991). [Supported by NIMH grant MH51015]


We have collected a sample of nearly 200 neurobiotin-filled cells from rats aged PND4 to PND12. A small number of these neurons (c10%) exhibit two axons. The prevalence of multiple axons increases as a function of age being more frequent in younger rats, but is unrelated to experimental group. All neurons seem to have morphologies consistent with that of granule cells. One axon typically arises from the soma while additional axons take origin from either proximal or distal dendrites. Examples of both axons originating from the cell body exist. Both axons may be mossy fibers regardless of their site of origin or one axon may be a mossy fiber and the second assume a distribution similar to an interneuron. In older rats, mossy fibers are seen to originate from proximal dendrites in the absence of a second axon from the soma. Similarly, cells with an axon arising from a distal dendrite and projecting within the molecular layer have been seen. These data suggest that some granule cells may express a mixed axonal phenotype during maturation and that some of these cells will maintain the atypical axon.

In adult transgenic mice, expressing B-50/GAP-43 in the adult olfactory system, using the olfactory marker protein (OMP) promoter, B-50/GAP-43 expression was directed to mature olfactory neurones, which do not normally express B-50/GAP-43. Mice bearing the OMP-B-50/GAP-43 transgene exhibited B-50/GAP-43 immunoreactivity in cell bodies, dendrites and axons of numerous mature neurones throughout the olfactory epithelium. This pattern of transgene expression was consistent with the action of the OMP promoter. We find that B-50/GAP-43 expression in mature olfactory neurones results in the formation of numerous hypertrophic OMP-positive primary olfactory axons with enlarged nerve endings. Confluent laser confocal microscopy on double-immunostained sections revealed that the mature olfactory neurones often termininated in dilated grape-like structures which were preferentially located on the rim of the glomeruli. Double-labelling with anti-tyrotype hydroxylase (TH) demonstrated that some OMP-positive olfactory neurones exhibited ectopic projections, between the TH-positive juxtaglomerular cells or associated with extra glomerular blood vessels. These observations were not observed in wild type litter mates and could be confirmed by Golgi staining of individual olfactory axon terminals. These data demonstrate that expression of B-50/GAP-43 in adult primary olfactory neurones in vivo has a direct effect on the morphology and projection territory of their axon terminals and supports a role of this growth-associated protein in nerve fiber formation.

789.11 ROLE OF HIGHLY CONSERVED 3' UTR SEQUENCES IN THE GAP-43 mRNA IN RNA STABILITY. N. J. Perrone-Bizzozero1, R. Thompson1, K-C. Tai1, V.V. Capsino1, D. T. Johnson1, K. L. Neve1, Dept. Biochemistry, Univ. New Mexico Sch. of Med., Albuquerque NM, 87131 and McLean Hospital, Belmont, MA 02178.

We have previously shown that the mRNA for the neural-specific growth-associated protein GAP-43 is selectively stabilized during neuronal differentiation (J. Cell Biol. 120, 1263-1270, 1993). Given the absence of known instability-conferring elements in this mRNA, we explored the role of highly conserved sequences in its 3' untranslated region (3' UTR) in mRNA stability. Between the rat and chicken GAP-43 mRNAs, the 3' UTRs show 78% of sequence identity, a level that is similar to the conservation of their coding regions. In mRNA stability assays in transfected rat PC12-N36 cells, both the rat and chicken mRNAs decayed with half-lives of about 3.5 h. The GAP-43 3' UTR was also found to destabilize the otherwise stable globin mRNA, with the half-life of the chimeric transcript comparable to that of the globin mRNA, suggesting that the 3' UTR is the major determinant of GAP-43 mRNA stability. The rat and chicken GAP-43 mRNAs were also tested in RNA cytosolic co-immunoprecipitation experiments. Both mRNAs bound equally well to the three GAP-43 mRNA binding proteins from rat brain. Also, >90% of proteins from chicken brain were able to bind rat GAP-43 mRNA sequences. Based upon these results, we propose that highly conserved sequences in the 3' UTR of this mRNA contribute to the control of GAP-43 gene expression via specific RNA-protein interactions. Supported by NIH (NS-30555, GM-08139) TSGH-NMDC, Taipei, and RAC-1090, UNM Sch. Med.

789.10 PROMOTER ELEMENTS CONTRIBUTING TO GAP-43 GENE REGULATION IN NEURONS. J. R. Weber, C.P. Hanes, and J.H. Pare,* Dept. of Neurobiology, Duke Univ., Durham, NC 27710.

Previous work in this and other labs has indicated that the 5' flanking region of the rat GAP-43 gene displays a significant degree of neural specific expression, and we now show in transgenic mice that 1 kb of this region can confer a significant degree of the temporal regulation of the GAP-43 gene. As one approach to elucidating the signaling pathways controlling GAP-43 expression in developing or regenerating neurones, we are now identifying individual transcription factor binding sites. We have focussed on a 386 bp subfragment of the 1 kb region that drives strong expression of a reporter gene in transiently transfected neurones from developing rat cerebrocerebral cortex, but has little or no activity in non-neuronal cell lines. Deletions within this 386 bp promoter indicate that a fairly small region located downstream of the TATA box consensus sequence is required for expression in neurones. Gel shift assays have identified three sequences within this fragment that bind nuclear proteins from developing rat cerebral cortex: an AP1 consensus sequence and two apparently novel sites, Cx1 and Cx2. Mutation of all three of these binding sites eliminates the majority of activity from the 386 bp promoter in cortical cultures. In contrast, gel shifts with liver nuclear extracts have shown a strong binding activity (Lvi1) which overlaps with, and would likely preclude binding to, Cx1 and Cx2. These studies have identified a small cluster of transcription factor binding sites, the AP1/Cx region, which is likely to play an important role in the regulation of the GAP-43 gene in neurones. Supported by NIH grant EY07397.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION VII

790.1 IMMUNOHISTOCHEMICAL LOCALIZATION OF CILIARY NEUROTROPHIC FACTOR RECEPTOR b EXPRESSION IN THE RAT. A. M. Mallatman, J. W. Vigers, L. Marks, R. Pfeiffer, and N. Lee, Department of Neuroscience, University of Florida, Gainesville, FL, 32610-0244.

Ciliary neurotrophic factor (CNTF) decreases the death of neurones induced by natural causes, axotomy or genetic mutations. Molecular cloning and heterologous expression studies indicate that CNTF produces most, and possibly all, of these effects by binding to a receptor referred to as CNTF receptor (CNTFR). Genetic "knockout" of this protein leads to significant developmental abnormalities. We used synthetic peptides corresponding to regions of the CNTFR to raise and affinity-purify anti-CNTFR polyclonal antibodies. The antibodies identify CNTFR in the adult and developing central and peripheral nervous systems. Specificity of these antibodies were found in the olfactory bulb and the cell bodies and dendrites of cranial and spinal motor neurones, central monoaminergic neurones, cerebellar purkinje cells and hippocampal neurones. Elevated levels were also detected in the axons of adult and developing peripheral nerves.


We investigated the presence of preponsins I and II (PPI, PPII) mRNA within the fetal rat brain, spinal cord and dorsal root ganglia (DRG). We also studied the presence of the alpha chain of the insulin receptor (IR) mRNA in the same tissues. Total RNA was obtained from 15,17 and 19 days gestational age fetal brain, spinal cord and DRG. PPII mRNAs served as positive control for PPI and PPII mRNA, and were detected in all tissues. Total RNA was subjected to reverse transcription template-specific PCR (RT-PCR) and RNAase protection assay (RPA) for PPI and PPII. IR was studied using RS-PCR. RS-PCR demonstrated a product of 381 base pair (bp) in brain, spinal cord and DRG in the 15, 17 and 19 days gestation that comigrated with the pancreatic product showing the presence of mRNA for PPI and PPI in these tissues. Semi-nested PCR products were then used in in situ hybridization with riboprobe detection by both anti-sense and sense digoxigenin and Rasl. The predicted fragments of 140 and 130 bp were obtained with Rasl for PPI, and fragments of 118 and 212 bp with Rasl for PPI. Rasl cuts only PPII and Rasl cuts only PPII. In solution hybridization using a 32P-cRNA for PPI or PPI, followed by NPA showed bands from the 15,17 and 19 days gestational age brain, spinal cord and DRG that comigrated with hybridized RNA from pancreas and with probe alone. RS-PCR also showed a fragment of 550 bp for IR as predicted within the brain, spinal cord and DRG for all ages studied. Fetal brain tissue was also prepared to localize insulin by electron microscopy (EM) and showed insulin immunoreaction within the endoplasmic reticulum (ER), Golgi, cytoplasm, and cell processes. This study showed that preponsins I, II and insulin, receptor are present within the fetal central and peripheral nervous system during nervous system development. Thus, the presence of the PPI and PPII mRNPs IR and insulinreceptor within the ER and Golgi strongly indicate the de novo synthesis of insulin within the central and peripheral nervous system.

IGFs are paracrine and autocrine neurotrophic factors, and both IGFBPs and their receptors are expressed in the mammalian spinal cord. IGFBPs have been postulated to play a role in regulating the survival and differentiation of neurons. However, little is known about the role of IGFBPs in the developing spinal cord.

Chromosomal Localization and Alternately Spliced Forms of Human Glial Cell Line-Derived Neurotrophic Factor (GDNF) and its Receptor, Ret. D. J. Choi-Lundberg, 1, 2, 3 D. A. Figlewicz, 1, 2 and M. C. Bohn. 1, 3 Depts. 1, 2, 3 Neurobiol. & Anat. and 4Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY 14620.

GDNF is a potent neurotrophic factor for lower motor neuron and spinal cord development. It is expressed in the spinal cord and has been shown to be upregulated after sciatic nerve injury. GDNF has been shown to be involved in the development and regeneration of spinal cord neurons.


In the present study we analysed the distribution of trkB and BDNF in the adult rat visual cortex, focusing on parvalbumin (PV) positive cells, the major contingent of GABAergic neurons. Using antibodies to trkB (Santa Cruz Biotech, CA) we have found staining in both pyramidal and non-pyramidal neurons. Double labelling experiments using antibodies to PV show that most PV positive neurons co-localized trkB. Pyramidal neurons positive for trkB were surrounded by PV labeled axons, whereas a small number of non PV neurons co-localized trkB. Pyramidal neurons positive for trkB were surrounded by PV labeled axons, whereas a small number of non PV neurons co-localized trkB. Pyramidal neurons positive for trkB were surrounded by PV labeled axons, whereas a small number of non PV neurons co-localized trkB. Pyramidal neurons positive for trkB were surrounded by PV labeled axons, whereas a small number of non PV neurons co-localized trkB. Our results suggest that trkB, which is expressed by pyramidal cortical neurons (Wetmore et al. Exp. Neurol. 109: 141-152, 1990), may act as postsynaptically derived neurotrophic factor or neuregulator of PV neurons of the adult rat visual cortex.

Supported by NIH RO 93/93 and BIOMED BMH-CT94-1378.

GDNF mRNA IS FOCALLY EXPRESSED IN DEVELOPING MOUSE LUMBAR SPINAL MARROW IN REGIONS WHERE AXONS FORM THE PLEXUS. D.F. Wright, 1, 2, 3 R.W. Gibbs, 1, 2, 3 D. L. Saunders, 1, 2, 3 J. H. Black, 1, 2, 3 L. P. M. Hamlett, 1, 2, 3 and L. C. Zhang, 1, 2, 3 Department of Neuroscience, 1, 2, 3 Neuroscience Research Center, 1, 2, 3 College of Medicine, 1, 2, 3 CSUSM, Washington University, St. Louis, MO, 63110.

The neurotrophic factor GDNF is a potent survival factor for motoneurons and is synthesized by Schwann cells and muscle. To further explore whether GDNF influences motoneurons during the early stages of peripheral innervation, we performed in situ hybridization on motoneurons of the developing rat hindlimb. A mouse GDNF cDNA was PCR-amplified to synthesize 32P-labeled riboprobes. Similar to rat and human, two splice variants were amplified, the longer fragment was used to generate riboprobes. At later embryonic stages (E14-E15), GDNF mRNA was detected in non-neuronal cells along peripheral nerve and developing muscle. However, at earlier stages (E10-E13), GDNF mRNA was not detected in non-neuronal cells along peripheral nerve and muscle. This suggests that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system. The results also suggest that GDNF may play a role in the development of the peripheral nervous system.
Prenatal Ontogeny of EGF Receptor and TGF alpha mRNAs in Rat Brain. H. J. Kempf1, K. Tatsukawa, R. J. Huseman, C. S. Galf1, D. Lusk2, and S. S. Numan2. 1 Dept. of Pharmacology, University of Salk, San Diego, California 92121; 2Dept. of Anatomy and Neurobiology, UCLA, CA 90095.

Neurotrophic factors seem to be expressed in several brain regions during development and adulthood. We have recently demonstrated the presence of a novel tyrosine kinase receptor (EGF-R) and its ligand TGF alpha in several brain regions during development and adulthood. The distribution of EGF-R and TGF alpha mRNAs have been demonstrated in several brain regions using in situ hybridization methods. The distribution of EGF-R and TGF alpha mRNAs is more widespread than previously thought. The distribution of EGF-R and TGF alpha mRNAs is more widespread than previously thought. The distribution of EGF-R and TGF alpha mRNAs is more widespread than previously thought.


Several epidermal growth factor (EGF) ligands, transforming growth factor-alpha (TGFa) and EGF, have recently been shown to promote survival and biochemical differentiation of neural populations from several brain regions. EGF and TGFa mRNAs in brain have been described, the presence of EGF-R ligands other than TGFa is currently unresolved. In the present study, in situ hybridization was performed in the rat brain to determine whether HB-EGF, a recently discovered member of the EGF family of mitogenic polypeptides, is expressed in the developing and mature central nervous system (CNS). Rat brain sections were processed for in situ hybridization localization of HB-EGF mRNA at neonatal and adult ages using a [35S]-labeled cRNA probe. The antiserum against HB-EGF cDNA was transcribed from a pBluescript KS vector containing a 258 base pair fragment derived from the EGF-R of cloned HB-EGF cDNA kindly provided by Dr. Judah Folkman, Scripps Nonva. Initial results have demonstrated HB-EGF mRNA hybridization within several cortical regions including the prefront cortex, hippocampus, and throughout the ventricular zone of the neocortex. In hippocampus, labeling was most prominent in the dentate gyrus. Several thalamic nuclei, including the ventrobasal complex, exhibited HB-EGF cRNA labeling. Brainstem, expression was detected in the dorsal raphe nucleus, area postrema, and caudal ventrolateral medulla. Dense hybridization was also present in the Purkinje cell layer of the cerebellum. HB-EGF mRNA levels were substantially higher neonatally than in adulthood suggesting that expression is developmentally regulated. These results raise the possibility that the novel EGF-R ligand HB-EGF may serve functional roles in select regions of the developing and adult brain. S.N. was supported by NS27180.

GDNF: A Member of a Gene Family Expressed in Many Subpopulations of Adult Brain Neurons. J. A. McEwen1, A. D. Dvoskin2, A. M. Kostokov2, C. Bader3, and P. Adashi2. 1Gene Therapy Center and Surgical Research Division, Lausanne University Medical School, Switzerland.

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GDNF: A Member of a Gene Family Expressed in Many Subpopulations of Adult Brain Neurons. J. A. McEwen1, A. D. Dvoskin2, A. M. Kostokov2, C. Bader3, and P. Adashi2. 1Gene Therapy Center and Surgical Research Division, Lausanne University Medical School, Switzerland.


Although extensively-applyed clinical neurotrophic factor (CNTF) exerts survival and differentiation effects on a variety of neuronal and glial cells in vitro and in vivo, the role played by endogenous CNTF is still incompletely understood. The roles of CNTF in the CNS may be to a large extent maintained by the presence of CNTF mRNAs in the CNS. The CNS is thought to be composed of two major regions, the PNS and its up-regulation after injury in the CNS has led to the proposal that CNTF may function as part of the response to damage in the nervous system. The levels and the sites and times of expression of CNTF in the CNS are key questions in the development and in adulthood are partially reversible. To resolve these questions we have introduced the leaC reporter gene into the CNTF coding region by homologous recombination in ES cells and expressed the reporter gene in several brain regions. In an attempt to correlate the CNTF promoter-driven leaC reporter gene expression with CNTF mRNA and protein expression patterns, we observed major differences in the CNTF gene expression between rat and mouse in certain brain regions. Experiments are underway to identify the cells expressing CNTF.
T90.15

IMMUNOLocalization of neurotrophin NT4/5 and its HIGH AFFINITY RECEPTOR TRK B in the RAT OLFACTORY BULB. L. J. Pali, J. N. Kott1, D. K. Kaplan1, L. E. Warren1, 3, Departments of Neurosurgical Surgery, 2, Biological Structure, 3, Psychology, 3, Univ. of Washington, Seattle, WA 98195; ABL Basic Research Program, NCI-FCRC, Frederick MD.

TRKB is the high-affinity receptor for the neurotrophin NT4/5 and exists in a truncated noncatalytic and full-length catalytic isoforms. Using immunohistochemistry, we examined the developmental localization of NT4/5 and TRKB in the rat olfactory bulbs (OB) of postnatal day 1, 5 (PN-1, PN-5) and adult animals. The OB is an ideal model in which to study the regulation process due to the unique receptive capacity of the olfactory nerves (ON) throughout adult life. Immunoreactivity for truncated and full-length TRKB remained moderate to strong throughout development in the ON and glomeruli (GL), while both full-length catalytic and NT4/5 immunoreactivity were non-existent at PN-1 and strong at PN-3 and adult ON and GL. As labeling for both growth factor and receptor is found in the same fiber population, these findings might suggest that these receptor subtypes in the OB derivate neurotrophic factor actions after retrograde transport back to the cell soma. The increased levels of NT4/5 and full-length catalytic TRKB during the later stages of development may play a role in an autocrine and/or paracrine regulated neuronal maintenance rather than a guidance mechanism. The observation that catalytic TRKB is expressed less strongly than truncated TRKB suggests that truncated TRKB may sequester excess NT4/5, thus limiting the diffusion of these growth factors. Supported by NIH grants NS06778 and a grant from NCNIH, under contract N01-10460.

T90.17

ACIDIC FIBROBLAST GROWTH FACTOR IS EXPRESSED BY BASAL FOREBRAIN AND STRIATAL CHOLINERGIC NEURONS. J.L. Biegon1, J.C. Leathtg2, R.C. Elliott1, and C.M. Gall1, 2, Dept. of Anatomy and Neurology, Univ. California, Irvine, CA 92717.

The basal forebrain cholinergic system is thought to be critical for the maintenance of cognitive abilities in both humans and rodents, and is susceptible to age- and injury-related degeneration. Recently, immunoreactive acidic fibroblast growth factor (AGF), a substance which reportedly supports cholinergic neurons, has been localized in basal forebrain. In the present study in situ hybridization was used to evaluate the distribution and degree of colocalization of mRNAs for AGF and the cholinergic marker choline acetyltransferase (ChAT) in rat forebrain. Neurons expressing AGF mRNA were colocalized with ChAT-positive neurons throughout all fields of basal forebrain including the medial septum/diagonal band region and the striatum. Cells labeled by the AGF cRNA also were distributed in regions not containing cholinergic neurons including lateral septum and several thalamic nuclei. Using double-labeling in situ hybridization, high levels of colocalization were observed in the medial septum, diagonal bands of Broca, magnocellular preoptic area and nucleus basalis of Meynert. In these we observed 50% of the cholinergic cells expressing AGF mRNA. In striatum few cholinergic neurons contained AGF mRNA and regional differences were observed. Specifically, among the ChAT mRNA-positive cells, 64% in caudate, 32% in ventral striatum (including nucleus accumbens) and 25% in olfactory tubercle expressed AGF mRNA. These data demonstrate substantial, regionally specific, patterns of AGF/ChAT mRNA colocalization and support the hypothesis that AGF is an autocrine neurotrophic factor for cholinergic neurons in basal forebrain and striatum. Supported by AG00538 to C.M.G.

T90.18


Previous work in our lab indicated that: (1) neurotrophic factors are expressed in cultured basal forebrain oligodendrocytes; NGF and BDNF mRNAs were detected by solution hybridization, and BDNF, NT3 and NT4/5 proteins by immunocytochemistry; and (2) the expression of BDNF mRNA can be regulated by KCI as a depolarizing signal. In order to further investigate the characteristics of the expression and regulation of neurotrophic factors under physiological conditions, and the effect of different neurotransmitters, enriched basal forebrain oligodendrocyte cultures were treated with 10 μM glutamate for 48 hours. In contrast to the effect of KCl, which increases BDNF mRNA expression, glutamate treatment caused a decrease of BDNF mRNA to nearly undetectable levels. These preliminary data suggest that different types of depolarizing signals regulate neurotrophic expression in basal forebrain oligodendrocytes via different mechanisms. To evaluate the expression of neurotrophin proteins in basal forebrain in vivo, immunocytochemistry has been performed to co-localize neurotrophins with the oligodendrocyte markers A2B5, MAG and MBP. Preliminary results revealed overlap of the anti-NT3 positive cells with anti-A2B5, suggesting the presence of NT3 protein in the basal forebrain oligodendrocyte precursors. Ongoing studies will determine whether NT3 is also co-localized with MAG and MBP in vivo. In sum, our results suggest possible mechanisms for regulating neurotrophin expression by depolarizing signals in vivo and the existence of neurotrophin proteins in basal forebrain oligodendrocytes in vivo.

T90.16

DEVELOPMENTALLY REGULATED EXPRESSION OF TRKA AND CHAT IN THE RAT CAUDATE-PUTAMEN. Y. Li, D. O. Chry, L. F. Reichardt and W. C. Mobley*, Department of Neurology and Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143.

TRKA, the receptor tyrosine kinase for NGF, is expressed in basal forebrain cholinergic neurons (BFCNs); its expression in development is regulated by endogenous NGF (Li et al., 1995, J Neurosci 15: 2888-2905). In the present work, we examined trka expression in the developing caudate-putamen. Using double immunostaining, trka was localized exclusively to caudate-putamen cholinergic neurons (CPCNs). Trka mRNA was first detected at PD 4 by in situ hybridization histochemistry. Northern analysis showed that trka mRNA increased steadily from a low level at PD 4 to the maximal adult level. The temporal pattern for the increase was similar to that for ChAT mRNA. By immunostaining, trka protein was detected at PD0. Western blot showed that trka protein increased during development and followed the pattern for the mRNA. Examining tyrosine phosphorylation of trka in caudate-putamen tritirates, we found that NGF activated these receptors at PD0 and that receptor activation was robust in older subjects. Infusion of NGF increased trka and ChAT mRNA, as well as the size of trka-positive neuron cell bodies. Our results indicate that trka expression is highly regulated in CPCNs; they point to a role for NGF in CPCN morphological and biochemical differentiation.

Supported by NIH grants NS24054 and AG08938.
791.1
FUNCTIONAL HETEROGENEITY OF GABA_A RECEPTOR-MEDIATED RESPONSES IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN MALE AND FEMALE NEONATAL RATS. L.L. Smith, A.S. Clark, J.A. Lally, and L. P. Henderson. 1Dept. of Physiol. and Biochem., Dartmouth Medical School, 2Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Gonadal steroids act during a critical period extending from late fetal stages to the approximate age of day 10 postnatal in inducing sex differences in the ventromedial nucleus (VMN) and medial preoptic area (POA) of the rat hypothalamus. GABA_A-mediated synaptic transmission within these regions has been shown to play a key role in the expression of adult female sexual behavior. We have characterized GABA_A-mediated responses in the VMN and POA of neonatal rats (PN0-PN20) by analysis of current elicited by rapid perfusion of the brain. The specific emphasis was placed on sex differences in the sensitivity to GABA_A receptors and on the patterns of spontaneous inhibition present in PN0-PN20 sex differences in the sensitivity to GABA_A receptors and on the patterns of spontaneous inhibition present in PN0-PN20 neurons. GABA_A-mediated responses are present in both sexes on day 10 but are sexually dimorphic with male rats demonstrating a shift in response to GABA_A agonists. We have used a rat version of the RNase protection assay to quantify mRNA for both forms of GABA in tissue microinjected from specific brain regions. Data are expressed as the ratio of GAD mRNA to that of a housekeeping gene, GAPDH. GAD mRNA was quantified in the arcuate nucleus (ARC), dorsal medial nucleus (DMN), median preoptic area (mPOA), CA1 region of the hippocampus, amygdala (AMYG) and the suprachiasmatic nucleus (SNC) at PN10 and PN15. We have demonstrated that the sex-dependent expression of mRNA of GAD is not only present in the arcuate nucleus but is also present in the median preoptic area and the suprachiasmatic nucleus. No sex differences were observed at PN10, whereas at PN15, GAD mRNA was significantly greater in the ARC of males and both forms of GAD mRNA were higher in the AMYG of males. There were no significant differences in other brain areas and no differences at PN25.

We conclude that varying levels of GAD expression during the critical period is involved in establishment of sexually dimorphic brain regions. Additional experiments will examine sex differences in adults and the impact of neonatal exposure to sex steroids on the development of the GABA_A receptor system.

791.2
SEX DIFFERENCES IN GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA CONTENT IN THE NEONATAL BRAIN. M.M. McCarthy1, D.R. Gattam, A.M. Davis and M. Saffel2. 1Department of Physiology, Univ. of Maryland, Baltimore, MD 21201

Although there are sex differences and steroid effects on the inhibitory neurotransmitter GABA in the adult brain, a role for this neurotransmitter in the sexual dimorphism of the neonatal brain has not been established. Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in GABA synthesis and controls the supply of GABA for both postsynaptic inhibition and neurotransmission. We have used a tissue-specific version of the RNase protection assay to quantify mRNA for both forms of GAD in tissue microinjected from specific brain regions. Data are expressed as the ratio of GAD mRNA to that of a housekeeping gene, GAPDH. GAD mRNA was quantified in the arcuate nucleus (ARC), dorsal medial nucleus (DMN), median preoptic area (mPOA), CA1 region of the hippocampus, amygdala (AMYG) and the suprachiasmatic nucleus (SNC) at PN10 and PN15. We have demonstrated that the sex-dependent expression of mRNA of GAD is not only present in the arcuate nucleus but is also present in the median preoptic area and the suprachiasmatic nucleus. No sex differences were observed at PN10, whereas at PN15, GAD mRNA was significantly greater in the ARC of males and both forms of GAD mRNA were higher in the AMYG of males. There were no significant differences in other brain areas and no differences at PN25.

We conclude that varying levels of GAD expression during the critical period is involved in establishment of sexually dimorphic brain regions. Additional experiments will examine sex differences in adults and the impact of neonatal exposure to sex steroids on the development of the GABA_A receptor system.

791.3

Endogenous testosterone is aromatized within brain cells to estradiol in males at critical periods during development. Females are protected from the effects of endogenous estrogens through the presence of alpha-teto protein. However, exogenous estrogens can function like testosteronne and masculinize the female brain. In this study the effect of estradiol on development of the dopaminergic system was studied. Male and female F344 rats were injected (i.c.) post-natal day 3 with either 1 pg estradiol benzoate or sesame oil vehicle. Animals were sacrificed between postnatal days 6-35. Brains were dissected and frontal cortex, hippocampus, and striatum removed and frozen. The dopamine D1 receptor was assayed in these tissues using [3H] dopamine as radioligand and the D1 agonist (a) SKF-81976 hydrobromide as the unlabeled competitor. Levels of [3H] dopamine binding to the D1 receptor subtype were decreased in cortex and hippocampus of both males and females receiving neonatal estradiol, relative to male control animals. Conversely, D1 receptor binding was increased in the striatum of estradiol treated animals. These results suggest that estradiol affects development of the dopaminergic system in a region-specific manner.

791.4
IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION HISTOCHEMISTRY OF CORTRICOTROPIN-RELEASING FACTOR(CRF)-CONTAINING NEURONS IN THE FEMALE RAT BRAIN. T. Fujikake, I. Akimura, T. Matsuda, T. Ishikawa and S. Nakamura. 1Dept. of Physiology and 2Dept. of Obstetrics and Gynecology, Yamaguchi University, Sch. of Med., Ube, Yamaguchi 755, and 2Dept. of Medicine, Tokyo Women's Medical College, Tokyo Japan.

Immunohistochemistry of CRF and in situ hybridization histochbesty were used to examine prenatal development of CRF-containing neurons in the rat brain. CRF mRNA was first expressed in the ventromedial hypothalamus and hypothalamic paraventricular nucleus at embryonic day 21.5 (E21.5) and was present in CRF-immunoreactive cells in comparable regions at birth. CRF immunoreactivity was first detected as early as E17. These findings indicate the presence of CRF-containing neurons in various brain regions during the prenatal period. We are further investigating the possibility of the coexpression of CRF with vasopressin in fetal brain neurons. (Supported by Grant in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan).

791.5

The rat adrenal gland (AG) contains ganglion cells able to synthesize nitric oxide, a neurotransmitter/neuromodulator involved in the control of adrenal secretory activity and blood flow. In the present study we analyzed the number and distribution of nitric oxide-producing adrenal neurons during postnatal development in the two sexes by means of NADPH-diaphorase (NADPH-d) histochemistry. Wistar albino rats were perfused at various postnatal ages and the AG processed to visualize the NADPH-d. While the AG of male rats was already well organized by the end of the fourth week in both sexes, a transient sex-related difference in the number of NADPH-d-positive neurons per AG is found during the second postnatal week (PG; male: 463 ± 35 vs female: 192 ± 6; P10: male: 473 ± 34 vs female: 360 ± 61; P15: male: 569 ± 43 vs female: 278 ± 62). If newborn male rats are killed daily, the number of NADPH-d-positive adrenal ganglion cells at P10 is significantly decreased in the adrenal cortex. The number of positive neurons at P10 is nearly doubled in the medulla. In conclusion, the present data demonstrate a transient sex influence on the number of NADPH-d-positive neurons in the rat AG, limited to the second postnatal week, and probably related to the perinatal difference in sex steroids in the two sexes. (Supported by MURST grants to AV)

791.6
ESTROGEN INDUCES AN INCREASE IN SINGLE CELL EXPRESSION OF PROENKEPHALIN mRNA IN THE VL-VMN OF YOUNG AND ADULT FEMALES. L.E. Rats. C. Gómez, A. Rodríguez1, J.L. González1, J.A. Jensen2, B.S. McEwen1 and A.C. Segura3. 1University of Puerto Rico, Physiology Department, and Neurosurgery Department; 2San Juan PR. 00936 and 3The Rockefeller University, NY, 10021.

Estrogen regulation of proenkephalin expression in the ventrolateral-ventromedial nucleus of the hypothalamus (VL-VMN) was studied in two age groups of female rats: juveniles and young adults. The juveniles were injected with oil or estradiol benzoate (40 mg/kg) at days 21 and 22 and sacrificed at day 23. The adult group consisted of intact and gonadotropin-primed rats that received oil or estradiol benzoate (40 mg/kg) at days 72 and 73 and were sacrificed at day 74. Sections of the VMN were studied by in situ hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Neuronal levels were determined by radiomoosays. Estrogen increased the amount of proenkephalin mRNA expressed per cell in the VL-VMN of juvenile females. We also observed an increase in the amount of cells expressing low levels of proenkephalin mRNA in these rats. In the adult, ovariectomy reduced proenkephalin mRNA expression, estrogen restored mRNA levels to those of intact females.
791.7

ESTROGEN INDUCES AN INCREASE IN SINGLE CELL EXPRESSION OF PROGENEKININ mRNA IN THE VL-VMN OF YOUNG BUT NOT ADULT MALE RATS. Z. Rivera1, M. Rivera1, J. A. Angelico2 and A. C. Segarra2. 1University of Puerto Rico, Physiology Department, San Juan; PR. 00936; and 2Biology Department, Hunter College, CUNY, NY, NY.

Estrogenic regulation of progenekinin expression in the ventrolateral-ventromedial nucleus of the hypothalamus (VL-VMN) was studied in two age groups of male rats: juveniles and young adults. The juveniles were injected with oil or estradiol benzoate (40 μg/kg) at days 21 and 22 and sacrificed at day 23. The adult group consisted of intact and gonadectomized rats that were injected with oil or estradiol benzoate (40μg/kg) at days 72 and 73 and were sacrificed at day 74. Sections of the VMN were studied by in situ hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Estrogen increased the amount of progenekinin mRNA expressed per cell in the VL-VMN of juvenile males. In the adult, neither orchidectomy nor estrogen treatment had an effect on progenekinin expression in the VL-VMN. These results and those of Romano et al. (1990) and Hammer et al. (1993) indicate that estrogenic regulation of enkephalergic expression in the VMN of male rats changes with age and duration of estrogen exposure.

791.8

WITHDRAWN

791.9

DIFFERENTIAL REGULATION OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P BY ESTROGEN IN RAT SENSORY NEURONS. S. Saraswat, J. A. Angelico, N. T. Talpalarpy and M. M. Olszynski. Dept. of Cell Biol. and Anat., Chicago Medical Sch., N. Chicago, IL.

Calcitonin gene-related peptide (CGRP) and substance P (SP) are expressed in a variety of neurons in the central as well as peripheral nervous system, including most small nociceptive neurons of the dural root ganglia (DRG). In regions of the nervous system that contain estrogen receptors (ER), such as pituitary and several hypothalamic regions, estrogen (E2) has been shown to augment CGRP levels and to downregulate SP levels. Recently, we have found that many of the small, but not the large-sized, DRG neurons express high levels of ER. Since many of these small neurons also express SP and CGRP, it was of interest to determine how hormonal status affects peptide expression in the DRG. Female Sprague Dawley rats were ovariectomized and either treated continuously with estradiol (E2) or with a subcutaneous implant of a silastic capsule filled with 17β-estradiol for 21 days (+E group) or were left untreated for that same period (ovx group). Lumbar and sacral DRGs were harvested and immunostained using a variety of polyclonal and monoclonal antibodies to CGRP, SP or ER. Results showed robust CGRP immunoreactivity (IR) in most small-sized DRG neurons, as well as in some medium to large-sized neurons; SP-IR was largely restricted to the small neurons. CGRP-IR in the DRG was markedly enhanced in the +E group relative to the ovx controls, but SP-IR appeared to be less robust in the +E group. This differential regulation of peptide expression in the DRG is consistent with findings in other ER containing neuronal populations. Studies have been recently initiated to explore the functional consequences of peptide modulation in the DRG by E2. Preliminary findings suggest that temperature/pain thresholds (tail flick response) may be influenced by gonadal steroid status.

791.10


Nurr1 (nerve-related orphan gene) and Ngfiib (nerve growth factor inducible) are two closely-related orphan receptors belonging to the steroid hormone receptor superfamily, which are both encoded by immediate early genes. The function of these two proteins is unclear, but in a recently published study the two orphan receptors have been shown to be able to modify retinoic signaling. Recent studies have indicated that they might be involved in apoptosis. In order to further elucidate the role of these proteins we have applied in situ hybridization histochemistry to localize the possible presence of nur1 and ngfiib mRNAs in the developing and adult mouse and rat CNS.Expression of nur1 mRNA was found in several regions during early development; this expression persisted throughout the pre- and postnatal period and was also found in many areas in the adult CNS. Nur1 mRNA was found in the olfactory bulb, several parts of the cortex, in the hippocampal formation, in the ventral segmental area and in the substantia nigra. In contrast to nur1, ngfiib mRNA expression was not found in the prenatals CNS. Ngfiib mRNA was first detected in newborn mammals. Moreover, the transcript was almost exclusively found in small groups of cells in the basal ganglia. Postnatally the ngfiib expression increased and in the adult brain ngfiib mRNA was found in many different regions, including the olfactory bulb, striatum, hippocampal formation and cortex. The interesting pattern of distribution of these two receptors imply important functions in the CNS both during development and in adulthood.

791.11

NGF-RECEPTOR IMMUNOCYTOCHEMISTRY PROVIDES EVIDENCE FOR INCREASED NEURAL FORMATIONS IN POSTNATAL FOREBRAIN CHOLINERGIC NEURONS OF RATS RECOVERING FROM EARLY HYPOTHYROID BRAIN RETARDATION. A. Panagiotou and F. Melanisi. Dept. of Physiology and Biochemicals, Univ. of Illinois, Urbana, IL 61801.

Plasma thyroid levels were suppressed in rats pups from birth by addition of PTU (propylthiouracil) in drinking water (1g/L). This neonatally induced hypothyroidism results in profound retardation of neuronal growth in brain regions. Recovery from this condition was initiated by withdrawal of PTU at 25 days of age. Animals recovering from hypothyroidism showed markedly increased growth rates in body and brain regions including hippocampus and dentate gyrus. Expression of low affinity p75 NGF-receptor (NG-F-R) is thought to be largely confined to cholinergic neurons of the basal forebrain (BF) which project extensively to forebrain areas. We have shown that compared to 25-day control rats, there is a significant elevation in NG-F-R levels in BF neurons of hypothyroid rats. BF neurons were stained immunocytochemically using a monoclonal antibody to NGF-R (192-195 IgG, Boehringer-Mannheim). Three weeks after PTU withdrawal, NG-F-R immunoreactive neurons of BF in recovering rats showed markedly increased neuritic outgrowth and branching as compared to age-matched controls. This neuritic sprouting was most evident in neurons of magnocellular BF nuclei. The above results suggest regulation of NG-F-R levels in BF cholinergic system by thyroid hormones may underlie the markedly increased growth rates in rat brains recovering from early thyroid retardation.

Support: NIH grant (GM07143) and UICU Research Funds

791.12

NEUROTROPHIC FACTOR EXPRESSION IN THE ANDROGEN-SENSITIVE BULBOCAVEOUSUS MUSCLE. J. Xu and N. G. Forget. Program in Molecular and Cellular Biology and Department of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) of rats innervate striated perineal muscles including the bulbocavernous (BC) and levator ani (LA). The survival of SNB motoneurons and their target muscles depends on androgen during perinatal development. As a result, the muscles and motoneurons persist in males, but degenerate in females. It has recently been demonstrated that ciliary neurotrophic factor (CNTF), can mimic some of the effects of early androgen in this system. CNTF prevents the death of SNB motoneurons and BC muscles that would normally occur in female rat pups. These observations raised the possibility that neurotrophic molecules might mediate some effects of androgen on the developing SNB system. We have used Northern analysis to begin to examine expression of neurotrophic factors and their receptors in the SNB system. The BC muscle of newborn male rats exhibited little or no expression of CNTF, but abundant expression of the α-component of the CNTF receptor (CNTFRα) and of TrkA, the high affinity receptor for the neurotrophin, brain-derived neurotrophic factor (BDNF) and NT-4. Expression of both CNTFRαs and TrkB was very low in adult BC, indicating developmental regulation of these messages. To determine whether the expression of neurotrophic factors or their receptors might be androgen regulated, rats were treated with testosterone propionate or hydroxyflutamide, a potent antiandrogens, from embryonic day 20 (E20) through postnatal day 4 (P4). Expression of CNTF, BDNF, NT-4, CNTFRαs and TrkB was then determined by Northern analysis on E20, P1 and P5 in the BC, LA, "thigh" muscle and lumbar spinal cord. (Supported by NIH grant HD33044-01 and the Whitall Foundation.)
792.1
EFFECTS OF PRENATAL PROTEIN DEPRIVATION ON CORTICOSTERONE LEVELS IN ADULT RATS. P.D. Bouder*, D.A. Kugler*, N.C. Cigler, and J. Kugler. Psychology Department, University of Texas, TX, 787126.

Prenatal protein deprivation in rats has been used to probe long-term consequences of early nutritional deprivation. Altered hippocampal morphology and electrophysiological functioning, as well as increased 5-HT and 5-HIAA levels in a number of brain areas have been found. Corticosterone (CORT) levels are known to be regulated by brain 5-HT as well as glucocorticoid receptors in hippocampus. As part of a broader study in which we examined the behavioral, morphological and neurochemical effects of prenatal protein deprivation, we examined whether there would be long-lasting changes in basal levels of CORT or CORT response to stress. Five weeks prior to breeding, 3D rats were fed either a 6% or 25% casein diet (Farlane Teklad, Madison, WI). All pups were cross-fostered to 25% dams who had given birth within the same 24 hour period, so that the experimental group was designated 6/25 and the control group 25/25. Pups were weaned at PND 25 and maintained on the 23% casein diet for the duration of the study. At PND 70-78 tail blood was obtained from a small group of rats at baseline, following 50 and 60 min of restraint, and after one hour recovery. CORT levels were measured by RIA (ICN Biomedicals). Both 6/25 (n=7) and 25/25 (n=7) animals showed a robust response to stress as seen by at least a 4-fold increase in CORT levels at 30 and 60 min. However, sample size was too small to determine if the 2 groups differed in their response to stress. At PND 85, rats were sacrificed by decapitation, brains were dissected and analyzed for CORT levels. 6/25 males (n=9) had significantly higher basal CORT levels (mean ± SEM, 213.3 ± 61.5 ng/ml) than 25/25 males (n=14, 70.2 ± 15.6 ng/ml, p=0.048). 6/25 females (n=10) had a trend toward increased CORT (313.5 ± 95.9 ng/ml) compared to 25/25 females (n=14, 206.2 ± 44.5 ng/ml, p=0.127). Thus, there appears to be a long lasting increase in basal levels of CORT in prenatal protein deprived rats.

792.2
EFFECTS OF CORN-FEEDING AND PROTEIN RESTRICTION ON DEVELOPMENT OF GABA-ERGIC CELLS OF THE CEREBRAL CORTEX IN S.R. Morgane*, S.R. Dupont, A. E. de la Fuente, Lilly México, IN, Mexico City, IN, Mexico. D.F. and P. Neimat, Instituto de Investigaciones en Ciencias del Alimento, de la Universidad Nacional Autonoma de Mexico, IN, Mexico City, Mexico.

Nutritional deficiency in early life affects the development of the central nervous system, there is a permanent deficit in cell number, and cell maturation is retarded in terms of axonal growth, myelination and enzymatic development. These changes are accompanied by a delay in the appearance of innate behavioral patterns, and which seem to be permanently impaired. The effects of undernutrition in early life have been recently studied in our laboratory in the following aspects: brain development, postnatal cell formation and development of GABAergic innervation of the sensorimotor cortex. Normal adult rats and their malnourished offspring (25% of normal rats) were fed for 6 weeks with 1) Normal diet (commercial diet for rodents, 23 % protein), 2) (hypobiotics + 8 % protein) diet on a chow- purina base and 3) corn-based diet (with 8 % protein; low tryptophan and lysine diet). The histologic studies were performed in the pups 1, 7, 14, 21, 30 and 60 days after birth. GABAergic elements were analyzed by GABA-immunocytochemistry. Radial distribution of cells and laminar numerical densities were calculated at each stage of development by photomicrographs systematically obtained from the total cortex thickness. Results of these studies showed that cortical thickness, total cellularity and the GABAergic cells were significantly reduced in the corn-fed group. The statistical significance of the difference was lower compared with the 8 % protein diet group. Results suggest that omission of essential nutrients from maternal diet has a greater inhibitory effect on postnatal brain development than that produced only by protein restriction.

792.3

The effects of protein malnutrition on GABAergic cell density (GAD positive cells) was studied in the fascia dentata and hippocampal formation of 30 and 90 days old rats. Five weeks prior to mating female rats were fed a 6% casein diet while control rats were fed a 25% casein diet. Malnutrition was established prenatally (6/25), postnatally (25/25), or both (6/25). At 30 days of age, the 6/25 malnourished group showed both a 31% significant (p=0.0008) reduction in the hippocampus, and a 39% significant (p=0.0001) decrease in the dentate gyrus GABAergic cell density. Conversely, at the same age, the 25/25 group displayed a 27% (p=0.01) increase in the fascia dentata GABAergic cell density. No significant differences were found at 90 days of age in hippocampal formation.

These data suggest that malnutrition affects the hippocampal formation morphology differentially. The increase in GABAergic cell density found at 30 days old in the 6/25 group indicates a delay in the maturation of hippocampal formation inhibitory circuitry. A severe decrease in GABAergic cell density was found in the 6/25 group in the fascia dentata and hippocampal formation. In the same group, however, a recovery was found in both structures in 90 days old rats. Supported by DGAPA IN-204093.

792.4

It has been demonstrated that chronic protein malnutrition delays the maturation of the mechanisms that regulates the vigilance states. This study was designed to evaluate the effects of protein malnutrition induced by a 6% casein diet in rats, instituted 5 weeks before mating and continued during gestation and into postnatal life to their offspring, after REM-sleep deprivation in a "conflict experiment", using an inverted dark-light cycle (12/12). A baseline (BL) recording day at 60 days of age was followed by one day of REM-sleep deprivation by the platform technique in control and malnourished rats, followed by three recovery days. We found using EEG power spectral analysis that the EEG relative power density of theta frequency band (4-7 Hz) was significantly increased in both light and dark phases of BL day one, during the first hour of dark phase of recovery day one and at initial hour of light phase of recovery day 2, and during first and last hour of light phase of day 3. Theta frequency band (7-12 Hz) was significantly reduced during first hour of dark phase of BL. This results suggest that malnutrition affects the theta system that regulates the theta rhythm. Supported by DGAPA IN-208494.

792.5
PRENATAL PROTEIN MALNUTRITION ALTERS HIPPOCAMPAL LTP MEASURES AT ALL STAGES OF DEVELOPMENT. J.R. Galler, J.R. Morgan*, J.D. Bronston, P.J. Austin-LaFrance and J.R. Galler. Center for Behavioral Development and Mental Retardation, Boston University School of Medicine, Boston, MA 02118 USA.

The ability of prenatal malnourished rats to establish and maintain long-term potentiation (LTP) of the perforant path-dentate granule cell synapse was examined in freely-moving animals at 15, 30 and 90 days of age. Measures of the population EPSP slope and population spike amplitude (PSA) were calculated from dentate field potentials obtained prior to and following tetanization of the perforant path. Significant enhancement of both measures was obtained from all animals of both the diet and control groups at 15 days of age. The magnitude of enhancement obtained from the malnourished group was significantly less than that of age-matched, well-nourished controls. At 30 days of age, PSA measures obtained from approximately 50% of malnourished animals showed no significant enhancement, while measures obtained from the remaining 50% did not differ from controls. EPSP slope measures for this age group followed the same pattern. At 90 days of age, PSA measures also showed a bimodal distribution, with 50% of malnourished animals showing a decline in PSA measures, while the remaining 50% did not differ from controls. These results indicate that gestational protein malnutrition has an enduring impact on hippocampal neuroplasticity. Supported by NIH/NINDIC Grant # HD 022339.

792.6
PRENATAL EXPOSURE TO MALNUTRITION BUT NOT COCAINE IMPAIRS ACQUISITION IN THE RADIAL ARM MAZE. J.S. Shumake*, P.I. Stalzty, J.R. Galler, and L. Jenkins. Center for Behavioral Development & Mental Retardation, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Maternal cocaine abuse is often associated with malnutrition. To explore the separate and combined effects of these two insults, a task was examined in the adult male offspring of female rats exposed to protein malnutrition (6% casein diet) and/or cocaine (30 mg/kg) for 5 wks prior to mating and during pregnancy. Control rats were fed a 25% casein or chow diet and saline-injected performed as deep-injection controls for all diet groups. Rats were tested using the 8-arm radial maze with 4 arms baited and were required to collect all 4 food pellets within 5 min to complete a trial. Subjects were tested for 1 trial/day until they reached the task. Acquisition of this task was completed when the rats attained the stringent performance criteria of obtaining 3 out of the 4 food pellets within their first 4 trials (while still completing the trial) over 3 consecutive days. The results showed a clear dissociation between the effects of prenatal cocaine and prenatal malnutrition. There were no significant effects due to prenatal cocaine on any measure in this procedure and there were no additive effects with prenatal malnutrition. However, prenatally malnourished rats showed impairment in two measures of acquisition: number of trials to criterion performance and task efficiency (correct arm entries/total arm entries x 100) when compared to controls. No differences were observed in the number of working or reference memory errors, suggesting that the insult produced a general impact on the acquisition process. These results suggest that prenatal malnutrition, but not cocaine, can impair the acquisition of a radial arm procedure with a stringent performance criteria is employed. Supported by NIH grant DA 07934.

Recent evidence indicates that prenatal nutritional deprivation may be one early environmental exposure that increases the risk of schizophrenia. In line with a neurodevelopmental model of SCZ, we investigated the effects of prenatal protein deficiency on growth, dendritic developmental milestones, and dopamine mediated behaviors in pre- and post-pubertal rats under dopaminergic agonists and antagonists. Five weeks prior to breeding, virgin female SD rats were fed either a 0% or 24% casein diet (Harlan Teklad, Madison, WI). Pups were weaned at 25 days of age and all were housed in a single cage with 25% dams who had given birth within the same 24 hour period so that the experimental group was designated M/25 and the control group 25/25. Pups were weaned at PND 25, and maintained on the 25% casein diet for the duration of the study. Weights were measured at birth, and approximately every third day thereafter. As expected, weights of 625 animals did not differ from 25/25 animals at birth or anytime thereafter. From PND 21-25, pups were assessed for the attainment of developmental milestones and reflexes. Surprisingly, 625 rats attained several developmental milestones (i.e. righting reflex, negative geotaxis, acoustic startle, ear unfolding andhurst response) before 21/25 pups. At PND 35 and PND 60, stereotypies were measured in rats receiving amphetamine (0.75 mg/kg, SC). At both ages, 625 females (n=9), but not males, had a trend towards increased stereotypy levels compared to 25/25 females (n=15), which reached significance only at PND 35. However, among males, 1/3 mg/kg, IP induced locomotion and haloperidol (1.0 mg/kg, IP) induced catalepsy did not differ between 625 and 25/25 rats at either age, though 625 females (n=9) showed a trend toward increased amphetamine induced locomotion at PND 30.

Our results suggest that prenatal protein deprivation alters the attainment of developmental milestones, and selectively increases some dopamine-mediated behaviors in post-pubertal, but not pre-pubertal rats.

NUTRITIONAL AND PRENATAL FACTORS


The manipulation of a single amino acid in pregnant laboratory rats may have an effect on their progeny. During the third trimester, Sprague-Dawley pregnant rats were given a TRP diet, and another five a 0.3TRP diet (control). In the preweaning period the first generation (F1) of rats given the TRP diet (n=35), significant delayed growth was noted in comparison to the growth noted in the control (n=23). In the postweaning to adult period (F0 control (n=35), significant added growth was noted in comparison to the growth noted in the control (n=23). In the postweaning to adult period (F2) rats, whose grandmothers (F0) were given 3TRP diet, revealed longer periods of postnatal overeating, higher circulating testosterone, and heavier body weights in the second litter. Surprisingly, F0 rats were noted to be more pronounced that those observed in the F1 rats. Phenotypic evidence may be interpreted as having a transgenerational effect as the result of a prenatal TRP diet. The OPRF guideline will be followed for care.*


It is now believed that infants dying of sudden infant death syndrome might have a subtle underlying immaturity of the brainstem resulting in a developmental process in a compromised intrauterine environment. In this study we have examined the effects of chronic placental insufficiency induced by unilateral ligation of the maternal artery at mid-gestation (term=66-68 days) in guinea pigs (n=29). The prenatal development of nuclei in the brainstem involved with cardiovascular control and swallowing was analyzed using stereological procedures and immunohistochemistry. A method was devised to enable the techniques to be performed in alternate frozen sections. The total number of neurons, area of neuronal soma and volume of the hypoglossal nucleus, showed no significant difference between control and compromised fetuses. There was a proliferation of reticulospinal neurones determined by immunofluorescence to glial fibrillary acidic protein, in the dorsal motor nucleus of the vagus, nucleus tractus solitarius (NTS) and around blood vessels throughout the brainstem. Immunohistochemical analysis of neuropeptides in the brainstem of control and reticulospinal neuropeptides in control animals, revealed a decrease in substance P (SP) immunoreactivity in the spinal trigeminal tract; a significant (p < 0.05) increase of 33% in the number of SP-positive neurons in the NTS and an increase in met-enkephalin (ME) immunoreactive fibres in the hypoglossal nucleus. These results show that although chronic intrapartum dehydration does not alter neurogenesis, at least in the hypoglossal nucleus, there is a proliferation of anterior neural crest and the expression of neurotransmitter/neuropeptide-related factors is affected in nuclei involved with cardiovascular control and swallowing.

Ethanol Alters the Content of Pituatory and Brain B-endorphin on the 20th Day of Fetal Life in the Rat. H. T. K. Bakay, D. J. G. Konno, D. M. Hospital Research Centre Verdun, Quebec Canada H4H 1R3.

At the time of birth and early postnatal life the total content and concentration of B-endorphin in the pituitary gland was lower, while the concentration of hypothalamic B-endorphin was higher in the fetal ethanol than control offspring. In the present studies the content of B-endorphin peptide in fetal pituitary and distinct regions of the fetal brain were measured on the 20th day of gestation when the offspring are not experiencing the effects of ethanol withdrawal. Rats were fed during gestation (a) with a liquid ethanol diet ad libitum; (b) were pair-fed to the ethanol fed animals with an isocaloric liquid diet; and (c) were fed water ad libitum. On the 20th day of gestation the fetal pituitary and distinct regions of the fetal brain (amphibons, frontal cortex, septum, arcuate nucleus, amygdala, hippocampus, ventral tegmental area, and central gray matter) were dissected and extracted in 0.1 normal HCL for estimation of the peptides. Results indicated a lower content of B-endorphin in the pituitary gland, and slightly higher content of B-endorphin in the brain regions, of the ethanol exposed offspring. These ethanol induced changes in the content of B-endorphin may alter the process of neurogenesis in the fetal ethanol offspring. Supported by a grant from the NIH.
792.13
PERINATAL OPIOID EXPOSURE AFFECTS CHOLINERGIC DEVELOPMENT IN THE RAT. S. E. Robinson,* Q. Mo, M. J. Wallace, J. D. T. Ory, and P. M. Konko. Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

The present study was performed to determine whether perinatal exposure to ful (methadone, M) or partial (buprenorphine, B) µ-opioid agonists delays the development of striatal cholinergic neurons. On day 7 of pregnancy, Sprague-Dawley CD rats were anesthetized with methoxyflurane and implanted s.c. with osmotic minipumps filled with M (9 mg/kg/day), B (1.5 mg/kg/day) or sterile water (W). Maternal weight, food and water intake were monitored throughout pregnancy. Within 24 h of birth, litters were culled to 10 and fostered to dams implanted with minipumps containing M, B or W, so that W/W, M/W, W/M, M/M, B/W, W/B or B/B in utero/postnatal exposure groups were obtained. New pups were implanted in dams on postnatal day (PD) 10. Choline acetyltransferase (ChAT) mRNA was measured by Northern blot analysis in the striata of 10- or 22-day-old male pups, using an α-32P dCTP-labelled 300 bp ChAT cDNA probe. The ratio of ChAT mRNA to 28S RNA was significantly decreased in 10-day-old pups exposed to either M or B perinatally. By PD 22, only pups with continued postnatal exposure to M had reduced ChAT mRNA. Therefore, M and B reduce expression of mRNA for ChAT, which may reflect a delay in development of striatal cholinergic neurons. No, expression of ChAT mRNA returns to control values by PD 22 in the B-exposure group and in pups after exposure to M is stopped. [This research was supported in part by NIDA grant 1R01 DA05274].

792.14
SUBSTANCE ABUSE DURING PREGNANCY IN A HISPANIC POPULATION: PREVALENCE AND EFFECTS ON PROGENY. N. Del Valle,†‡ Z. Rivera, † J. A. Capiller,‡ M. P. Casado† and A. C. Segura.†† University of Puerto Rico, Psychology Department† and Municipal Hospital, Department of Pediatrics‡ Medical Center, San Juan, Puerto Rico 00936.

A sample of newborn children in the San Juan area Municipal Hospital was taken for a prospective longitudinal study. Each week, ten neonates, 5 males and 5 females, of less than 24 hrs were randomly selected to participate in the study. Urine samples of the newborn were collected and assessed by radioimmunoassay for cocaine and nicotine. Birth parameters such as birth weight, length, head circumference and fontanel size were recorded. In our sample, no neonates tested positives for morphine, 6% tested positive for cocaine and 14% tested positive for nicotine. A decrease in birth weight and length was observed in neonates whose urine tested positive for cocaine but not in nicotine-positive neonates. No effect was observed in head circumference nor in fontanel size in these nicotine and cocaine positive neonates. We also observed a sex difference in birth weight and length; males being larger and weighing more than females. No sex difference was observed in head circumference nor in fontanel size. We are currently in the process of establishing nicotine and cocaine urine cut-off values in neonates.

792.15

We have reported that prenatal stressful treatments such as injections, or glucocorticoid administration affect the development of serotonergic neuronal system in rat brain. On the other hand, prenatal nicotine exposure via maternal injections is a treatment inducing hypothermia and ischemia to fetus, that is mimetic to the effect of maternal tobacco use. Some studies indicate that effects of nicotine itself can be discriminated from overall effects of nicotine exposure by using injections. In the present study, we investigated effects of maternal nicotine injections(inj.) or infusions(inf.) on monoaminergic neuronal developments including serotonergic system. Pregnant SD rats were exposed to nicotine via injections twice a day (6mg/kg/day) or infusions by implanted osmotic minipumps (flow rate: 6mg/kg/day) from gestational day (GD) 4 to GD 20. In the midbrain-pons-midulla (M-P-M) and the forebrain of rat pups on postnatal day (PD) 7 and PD 15, NE, DA, DOPAC, HVA, 5HT and SHAA were measured by HPLC with ECD. Data were analyzed by two-way ANOVA (factors of treatment [inj. or inf.] and nicotine). Main significant changes are summarized below. In M-P-M, effects of inj. treatments were increases in NE, DOPAC, HVA and SHAA. Effects of nicotine were decrease in HVA and increase in 5HT. In forebrain, effects of inj. were increases in NE, DOPAC, HVA and SHAA, and decreases in DA and 5HT. Effects of nicotine were increase in DA and decrease in DOPAC. In addition, these changes in M-P-M and forebrain were dominant in PD7 and PD15 respectively. Our results indicated that nicotine exposure affected not only dopaminergic system but serotonergic system while inj. treatment was likely to affect various aminergic systems.

792.16
SEX DIFFERENCES IN BODY WEIGHT EFFECTS OF PRENATAL NICOTINE IN RATS. S. M. Nespor, E. J. Poole, M. A. Rahman, Y. Tashib and N. E. Gruntzer. Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799; and Dept. of Pharmacology, Coll. of Med., Howard University, Washington, DC 20059.

Maternal cigarette smoking during pregnancy results in decreased infant birthweight. These effects are consistent with the inverse relationship between cigarette smoke and body weight and between chronic nicotine administration and body weight. The effects of smoking on body weight in adults result from effects of nicotine on energy intake and energy expenditure and may involve effects of nicotine on plasma and hypothalamic insulin levels. The present experiment examined effects of prenatal nicotine exposure on the body weights and blood and hypothalamic insulin levels of Sprague-Dawley rat pups during the first five weeks post partum. Twenty-four pregnant dams received nicotine (6 mg/kg/day) or saline by osmotic minipumps, during days 4-22 of the 22-day gestation period. Body weights for the 118 male offspring and 104 female offspring were measured 18 days, 24 days, 30 days, 34 days and 36 days post partum. Blood and hypothalamic tissue from the offspring were assayed by radioimmunoassay for insulin levels. Nicotine significantly and consistently reduced the body weights and the plasma and hypothalamic insulin levels in male offspring. Nicotine slightly reduced the body weights and the plasma and hypothalamic insulin levels in male offspring. In addition, for females and males, the body weights correlated positively with the plasma and hypothalamic insulin levels (r=0.01). These results are consistent with previous reports that nicotine affects body weight and insulin levels and that females are more sensitive to these effects of nicotine.

792.17
IN UTERO EXPOSURE TO PAROXETINE LEADS TO ALTERED PHYSICAL AND MOTOR DEVELOPMENT. J. H. Patton,* C. H. Rauen, J. L. Langloë. Neurosciences Program, Baylor University, Waco, TX 76799-7334.

Previous research in our laboratory has demonstrated that in utero exposure to Fluoxetine can cause morphological and behavioral teratogenic effects (Stanford & Patton, Pharm. Biochem. & Beh., 1993, 45, 959-962; Patton, Langloë, & Rayer, Poster Presented at SWPA, 1995). To test the generalizability of these effects, we exposed dams to approximately seven times the maximal human dose of paroxetine on a mg/kg basis (4.66 mg/kg) via oral gavage beginning on day seven of gestation and ending the day of birth. Control animals received distilled water in similar volume by gavage during the same time period. On day three, all litters were culled to eight members, and individual pups were weighed, measured, and sexed for identification. Behavioral assessment began on day five. Paroxetine exposed litters had an increased number of stillbirths and neonatal deaths (Stillbirths: t = 2.26, p = 0.037, df = 17; Neonatal deaths t = 2.49, p = 0.021, df = 17). In utero paroxetine exposure had no effect on weight and length, nor upper and lower incisor protrusion time; paroxetine exposure did, however, result in earlier eye openings (F1,122 = 23.043, p < 0.001). Although paroxetine pups performed similarly to controls on surface righting, paroxetine exposed pups performed faster on tests of negative geotaxis (Lazene: F1,122 = 4.31, p = 0.039; Trials to Criterion: F1,122 = 4.516, p = 0.036). The paroxetine pups also required fewer trials to reach criterion on cliff avoidance (F1,122 = 3.248, p = 0.050). Combined with our previous work with Fluoxetine, results of this study suggest caution in the administration of SSRIs during pregnancy.
783. 1
IDENTIFICATION OF CORTICOSTERONE-RESPONSIVE GENES INVOLVED IN HIPPOCAMPAL DEGENERATION. E. Yodogawa*, J. D. long, L. S. Buxton and E.R. de Kloet, Leiden/Amsterdam Center for Drug Research, Division of Medical Pharmacology, PO box 9503, 2300 RA Leiden.

Fluctuating levels of adrenal corticoids play a pivotal role in the viability of hippocampal neurons. To examine, neuronal as well as excitotoxic corticosteroids may result in the neurodegeneration of hippocampal neuronal circuits and consequently result in death and in adaptation to stress.

To investigate the molecular mechanisms underlying corticosteroid-mediated neurodegeneration in the hippocampus we have applied the differential display technique to compare the hippocampal expression profile of four groups of animals. We have used ADX rats and compared those with sham-operated animals. Furthermore, as glial cells are an important additional factor in hippocampal neurodegeneration, kainic acid was administered to ADX rats and to sham operated rats. The display of approximately 1800 spots identified the identification of two differentially expressed products between the ADX and sham-operated group and four between the sham-operated and kainic acid treated animals. The comparison of the ADX, sham and kainic acid treated groups with the ADX/kainic acid group, however, reveals 18 differentially expressed genes. These results indicate that the main mode of corticosteroid receptor-controlled gene expression in the hippocampus is interaction with other transcription factors (e.g. CREB, AP-1) and not by binding to hormone responsive elements of corticosteroid-specific genes. Furthermore, because corticosterone in levels in the kainic acid-treated group were such that only the high-affinity MR was occupied, we suggest that the modulating effect of corticosterone on kainic acid-induced expression is mediated by the MR and not by the GR.

In conclusion, we will present data on the nature of some of the differentially expressed genes and discuss their possible role in hippocampal neurodegeneration.

783. 3
RELIABILITY AND VALIDITY OF THE PHYSICAL DISECTOR. G.J. Popken, P.B. Fawcett* Curriculum in Neurobiology and Dept. of Physiology University of North Carolina School of Medicine Chapel Hill, NC 27599.

The analysis of many neurobiological phenomena requires accurate and reliable estimates of neuron number. The physical dissector (Sterio, 1984) has been offered as an unbiased and efficient means to estimate neuron number. The dissector method provides estimates of neuron number based on a small sample of cells, providing efficiency, but at a potential cost in reliability. In this study the reliability and validity of the dissector method were investigated using frog dorsal root ganglia. Effects of variables related to tissue orientation, volume estimation and sample size were also considered. Recommended protocols for data acquisition by means of the physical dissector were found to introduce variability in estimates of neuron number ranging from 0.2 to 5.9%.

The variability could mask experimental effects or introduce spurious trends. Though recommended sampling protocols can lead to unreliable estimates, statistically reliable and valid estimates were achieved with this method when the sample size exceeded the recommended value by 3-6 times and when careful attention was paid to tissue preparation and volume measurements. Dissector estimates were consistently lower than empirical estimates for ganglia cut parallel to the long axis of the dorsal root but not for ganglia cut transverse to this axis. Application of the physical dissector thus requires empirical validation and careful consideration of variables that may be specific to the particular experimental situation. Supported by grants NS16030 and NS14899.

783. 5

Certain apoptosis-related genes, including bcl-2, bcl-x, and bax, share homology at the amino acid level in the BH1 and BH2 domains, through which they also interact. As part of a search for novel members of this family, we studied their expression in retinal ganglion cells after axotomy. We designed degenerate oligonucleotide primers to the BH1 and BH2 domains, reducing multiplicity by eliminating nucleotides specific to human sequences. Total RNA was isolated from Long-Evans rat retinas at 1 and 4 days after intraretinal crush of the ipsilateral optic nerve; the contralateral retinas were used as a source of control RNA. Reverse-transcribed (RT) cDNA was amplified by the polymerase chain reaction and the products separated with a 1.5% agarose gel.

A single band of the predicted 160 bp length was seen in the control retinas; this monotonically decreased in retinas undergoing axotomy. There were no differences in BH1 (cytobolin protein); I-F-negative controls demonstrated no banding. This suggests that expression of one or more members of this family decreases after axotomy, which may correlate with retinal ganglion cell apoptosis in this setting. We are currently testing this hypothesis with in situ hybridization (Supported by NIH EY00340, Research to Prevent Blindness, and American Health Assistance Foundation.)

783. 6

Overproduction of cells and subsequent elimination of the excess are common features of nervous system development. In a typical brain region about twice as many neurons can be found in development than in adult life. However, as many investigators have already indicated, the number of 5-10% of neurons could be higher than those figures suggest, since in many neuronal populations death begins when new cells are still appearing. A realistic estimate of the magnitude of cell death is missing for the lack of precise methods for assessment. However, by labelling cells according to their time of genesis with 5-bromo-2′-deoxyuridine (BrdU), we have been able to follow cells born in limited intervals of time and obtain a minimal estimate of the number of dying neurons in the rat retinal ganglion cell layer. Surprisingly, our data suggest that at least 5 times as many ganglion cells die than commonly believed, and a similar figure is obtained for displaced amacrine cells.

783. 7
SERUM RESCUES NEURONS FROM HYPEROSMOTIC-INDUCED PROGRAMMED CELL DEATH. C. Dieckman* and E.J. Feldman. Department of Neurology, University of Michigan, Ann Arbor 48109.

SH-SY5Y neuroblasticoma cells are a cloned cell line which ultrastructurally resembles developing neurons. These cells are a good model system in which to study the potential mechanism by which neurotrophins secondary to hyperglycemic, hyperosmotic exposure, as seen in diabetes. In the current study, we determined if serum could rescue neuronal cells from hyperosmolar induced growth arrest.

SH-SY5Y cells (1 x 10^6 cells/cm^2) were rinsed and plated directly in serum-free media a 5, 20, 50, 100 and 300 mM mannitol. Cell number was measured the following day and 3 by 3 colorimetric assay which detect reductions of the tetrazolium salt MTT. By day 2, 20 mM mannitol had significantly decreased SH-SY5Y cell number. The extent of growth arrest over time correlated with the severity of hyperosmotic exposure and was maximal at 300 mM mannitol. Serum rescued SH-SY5Y cells from glucose-induced growth arrest in a dose-dependent fashion with significant improvement in cell number within 24 hrs.

Programmed cell death (PCD), or apoptosis, is an active process which occurs when an essential signal is withdrawn or a non-signal is introduced. In normosomolar media, withdrawing serum from SH-SY5Y cells did not precipitate PCD measured by flow cytometry. In contrast, approximate 19, 40, 60 and 80% of cells undergo PCD in serum-free media made hypotonic in 300 mM mannitol after 24, 48, 72 and 96 hrs respectively. Addition of serum rescued cells from PCD; indeed, only 0, 16, and 20% of cells underwent PCD at 24, 48, 72 and 96 hrs respectively after hyperosmotic exposure in the presence of 10% serum. Rescue was serum dose dependent and initially observed at 0.9% serum. Collectively, our results imply that a factor in serum can act as a neuroprotective factor by rescuing cells from PCD. Sponsored by R29 NS32843 and an award from the Juvenile Diabetes Research Foundation International, Number 194130.
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The olfactory molecules are produced in the olfactory epithelium. The olfactory neurons contain a pattern similar to control cells for the majority of protein product analyzed. One single peptide binding of 120kd of molecular weight and an acidic isoelectric point showed an increase in 32P labelling triggered by shifting extracellular potassium from 25 to 3 mM. We followed the kinetic of the phosphorylative event from 0 to 3 hours after the triggering of apoptosis. The increase in 32P incorporation in the 120kd protein occurred in the first 20 min, a plateau was kept up to 1 hour and a slow decrease leading to basal control level was observed in the second hour of the apoptotic process. The serine threonine kinase inhibitor staurosporine selectively prevented the low potassium induced phosphorylation of the 120kd protein. The phosphate blockers okadaic acid induced an overall augmentation of the protein phosphorylation including the 120kd Peptide.

973.11

MULTIPLE CED-3 AND CED-9 HOMOLOGUES ARE EXPRESSED IN THE MURINE NEURONAL SYSTEM: A.D. Pandey*, J.L. Ellison, and W.D. Frazier. CBSD. Dept of Neurology. Washington University School of Medicine, St. Louis, Mo. 63110

Recent investigations in vivo and in vitro have confirmed the central importance of ced-3 and ced-9 homologues in the regulation of neuronal survival. However, each of these families is complex and appears to contain many interesting members, all of which are expressed spatially and temporally in patterns appropriate to regulate neuronal survival is unclear. We show here, using in situ hybridization, that the survival promoting ced-9 homologues (bcl-2 and bcl-x), the death promoting ced-9 homologues (bax and bad) and the bcl-2 binding protein (BAG-1) are differentially expressed in the murine peripheral and central nervous systems both during development and in adulthood. Similarly, the ced-3 homologue, med-2, is expressed by virtually all neurons at all developmental stages. In contrast, the ced-3 homologue ICE is not expressed in the murine nervous system.

There are, however, some developmental differences in expression patterns between the various family members. Temporally, mRNA for all of these molecules appears to decrease as animals mature, except for BAG-1. BAG-1 is expressed at low levels in embryonic mouse brain, then increases during early postnatal stages and into adulthood. In maturity, both ced-3 and ced-3 family members exhibit some cellular specificity in that expression is much more intense in neurons than in glial cells.

These results indicate that ced-3 and ced-9 homologues are expressed in the murine nervous system, underscoring their central importance to regulating both naturally occurring neuronal death during development as well as neuronal survival in adult animals. These results also suggest that programmed cell death in all neurons may be regulated by similar intracellular mechanisms.

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DENDRITIC APOPTOSIS: A NEW MECHANISM FOR RESTRICTED NEURONAL DEATH. M. Segnitz*, E.D. Churchway and R. Lindsay. Dept. of Psychology & Neuroscience, University of Iowa, Iowa City, 52242

While studying calcium concentration changes in Purkinje cell dendrites in vitro using calcium dependent fluorescent dyes, we noticed that certain manipulations resulted in the death of well defined dendritic segments. Upon further study, we found that ionophoresis of glutamic acid could trigger local release of calcium from intracellular stores even in the absence of extracellular calcium and in conditions where dendritic spikes were totally absent. When the ionophoresis injection was continuously repeated on the same site (6 to 8 times), the calcium concentration increased became prolonged and a lack of dendrite response to further ionophoresis was noticed. In order to determine whether the calcium concentration changes observed were triggered by activation of a metabotropic glutamate receptor, we treated the neurons with the specific IP3 dependent calcium release from intracellular stores, we repeated these experiments after intracellular injection of bebrin (500 mg/ml). This protein prevented the secondary release of intracellular calcium during glutamatic ionophoresis. We further observed that following glutamate ionophoresis to a given dendritic branch, the large release of intracellular stored calcium did not, necessarily, spread to other branches within the period observed in a slice condition. These results are consistent with the local release of calcium from intracellular stores may, under conditions, produce apoptosis of particular dendritic branches rather than the whole cell. There is evidence from neuropharmacology, especially in aging subjects, that such dendritic apoptosis is one of the main determinants of gray matter loss as opposed to the actual loss of whole neurons. Supported by NIA AG05480.

973.12

GENES ASSOCIATED WITH APOPTOSIS ARE EXPRESSED BY DYING MOTONEURONES AFTER NEONATAL AXOTOMY. R.W. Gerber*, J.L. Ellison, J.M. Johnson Jr. and W. D. Frazier. CBSD. Dept of Neurology and Molecular Biology, Washington University School of Medicine, St. Louis, Mo. 63110

Molecular mechanisms underlying neuronal programmed cell death are being clarified by the identification of genes which are associated with apoptosis in vitro. In order to assess the significance of these genes in an in vivo system, we asked whether acutely and chronically after axonal injury, motoneurons demonstrate evidence of apoptosis. We have used the chick embryo spinal cord model system and have assessed apoptosis in cultured neurons using TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling), a method that allows rapid and sensitive detection of DNA breaks.

This method utilizes a double label immunohistochemical reaction which is facilitated by a terminal deoxynucleotidyl transferase enzyme. The following components are used: DNA labeled with biotin, a fluorescein labeled anti-DNA antibody, and a peroxidase labeled streptavidin. We have examined cultured motoneurons from the chick embryo spinal cord to determine if apoptosis can be detected after axotomy.

We have observed that, following axotomy, motoneurons which are destined to degenerate rapidly display the characteristic features of apoptosis. These include nuclear condensation, pyknosis, chromatin fragmentation, and internucleosomal DNA degradation. These findings suggest that the process of programmed cell death in motoneurons after axotomy is similar to that observed in other cell types. Supported by NIA AG05480.

We have previously reported that neuronal axonized motoneurons which overexpress Bcl-2 were protected against neuronal death for a period of 7 days. Using the TUNEL technique, we have now observed that following facial nerve lesion in two days old wild type mice motoneurons die by apoptosis within 72 hours. In contrast, in transgenic pups in which apoptosis was not detected in lesioned facial motoneurons during this same period.

Furthermore we have examined the long-term survival of these lesioned facial motoneurons overexpressing Bcl-2. Twelve weeks after the lesion in two days old pups, 74% (n=3) of facial motoneurons survived axotomy in transgenic mice (when compared to the control litter). The same results were obtained for both type mice all lesioned motoneurons had died during the first week after the lesion. The mean diameter of control motoneurons was 26 μm whereas lesioned surviving motoneurons were smaller (13 μm, p<0.05). The nuclear size of control motoneurons was 13 μm and 9 μm in lesioned ones (p<0.05).

These results suggest that Bcl-2 confers long-term survival to the lesioned facial motoneurons. Moreover, this survival may be independent of neurotrophic factors since we have not observed facial nerve regrowth at the level of the facial musclecuture. However, ipsilateral to the lesion, facial nerve axons were always present in the portion of the facial nerve travelling in the brainstem of transgenic mice.

Bcl-2 PREVENTS OXYGEN-INDUCED APOPTOSIS IN PC12 CELLS. *T. Kobayashi, Y. Enido, T. Okita, N. Sato, Y. Uchihara*, and H. Hatanaka. Institute for Protein Research,Osaka Univ., 3-13 Yamadaoka, School of Medicine, 2-2 Yamadaoaka, Suita, Osaka 565, Japan.

The brain is one of the most energy consuming organs and exclusively dependent on the aerobic energy metabolism using oxygen and glucose. Thus the neurons are always exposed to oxidative stress in their long lives. It has been reported that the oxidative damage was deeply related to Parkinson’s and Alzheimer’s diseases. It is therefore important to investigate the mechanisms by which oxidative damage brings about the neuronal death. We have reported that apoptotic neuronal death was observed when embryonic rat hippocampal neurons were cultured in a 50% oxygen atmosphere (Hatanaka et al., 1993). This study the mechanism of oxygen-induced neuronal apoptosis in molecular levels, we established the system using PC12 cells. When cultured in a 50% oxygen atmosphere, PC12 cells largely died within 3 days. This cell death was prevented by protein and RNA synthesis inhibitors and the chromatin condensation was observed in cells grown in a 50% oxygen atmosphere. These results suggest that this cell death is mediated by an intracellular active death program, so called apoptosis. The high concentrations of potassium ([K+]o=26 mM) in culture medium also inhibited oxygen-induced apoptosis in PC12 cells.

To characterize the oxygen-induced apoptosis further, we used PC12 cells overexpressing the proto-oncogene bcl-2, which has been reported to prevent apoptosis in various types of cells. A large number of the bcl-2- transfected cells survived in a 50% oxygen atmosphere for 3 days in contrast to those transfected with the control vector. These results strongly suggest that Bcl-2 prevents oxygen-induced apoptosis in PC12 cells.

This system should be useful for analyzing the molecular mechanisms of oxygen-induced neuronal apoptosis in detail.

Cajal-Retzius cells are considered the principal cell type of neocortical layer I. They are absent during fetal life, but only a subpopulation survives into adulthood. We analyze here the distribution and morphology of neurons in layer I in histologic and cytologic areas (primary sensory, motor and association cortices) of 10 human brains aged 27-96 years, by using calbindin (CB) and calreitin (CR) immunohistochemistry and NADPH-diaphorase histochemistry. Like the fetal forms, adult Cajal-Retzius cells have a long horizontal axon and dendrites restricted to layer I. They occur in all areas examined, but show a topographical preference for the bottoms of the sulci. Most Cajal-Retzius cells are CR-immunoreactive (ir), but a few ones express CB. A subgroup of Cajal-Retzius cells, located deep in the sulci around entering blood vessels, is moderately NADPH-d positive. A second class of large cells in layer I is CB-ir. These neurons, observed in the prefrontal and non-auditory temporal areas, have long smooth dendrites descending to layer III, and an apparently local axon in layer I. Their similarity with CB-ir neurons in deeper layers suggests that they do not belong to the group of Cajal-Retzius cells, but rather share the morphology, and possibly the developmental history, of neurons derived from the cortical plate.


We studied the development of layer IIIC pyramidal neurons in the region of Brodmann's area 9 of the human prefrontal cortex (PFC) in rapid Golgi stained sections using a 3-dimentional model developed at the Netherlands Institute for Brain Research. Quantitative analysis was performed on basal dendrites of 25 subjects ranging from newborn up to 91 years. Research questions were: which phases of dendroplaological development can be detected? Is dendritic growth a continuous process or is there a period of development with a faster growth? Is there a correlation between dendritic growth and synaptic structures? In addition, a temporary exuberant number of spines on those pyramidal cells was detected during the period of 3-6 years (Poteocki et al., 1995). In this study we found that the postnatal development of layer IIIC pyramidal neurons was characterized by an early period of rapid growth followed by a slower growth phase, which continued until adulthood. The results of our study suggest that layer IIIC pyramidal neurons undergo a period of rapid growth before reaching a mature state, similar to other neuronal populations in the brain. The finding that layer IIIC pyramidal neurons display an early period of rapid growth is consistent with previous studies that have suggested that these neurons play a role in the regulation of motor behavior. The results of our study also suggest that the rapid growth phase of layer IIIC pyramidal neurons may be related to the development of connections with other neuronal populations in the brain. The findings of our study also suggest that the rapid growth phase of layer IIIC pyramidal neurons may be related to the development of connections with other neuronal populations in the brain. The findings of our study also suggest that the rapid growth phase of layer IIIC pyramidal neurons may be related to the development of connections with other neuronal populations in the brain. The findings of our study also suggest that the rapid growth phase of layer IIIC pyramidal neurons may be related to the development of connections with other neuronal populations in the brain.
CEREBRAL CORTEX AND LIMBIC SYSTEM IV

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995

**T94.5**
SEX DIFFERENCES IN RESTING CEREBRAL GLUCOSE METABOLISM IN MONKEYS. M. J. Raleigh, W. P. Melega, S-C. Huang, S. Cherry, Michael T. McGurre, and M. E. Phelps. Deps. of Psychiatry and Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024-1759.

This positron emission tomography (PET) study examined the relationship between sex differences in local cerebral metabolic rates for glucose (LCMRglc) and the emergence of gender differences in behavior in developing vervet monkeys. We documented LCMRglc in nine regions of interest (ROI) in conscious monkeys. The seven male and eight female subjects ranged from 2 to 14 months and lived in complex, sex-specific social groups. Sex differences in aggression, affiliation and play were not present prior to six months of age but became prominent between six and 12 months of age. Similarly there were no sex differences in LCMRglc in any ROI before subjects were six months old. However, between six and 12 months of age, females had higher LCMRglc than males in the orbitofrontal, dorsolateral frontal, temporal, and parietal cortex by 21%, 23%, 25%, and 23% respectively. By contrast, there were no sex differences in LCMRglc in the visual cortex, primary motor cortex, thalamus, cerebellum, or brainstem. Thus sex differences in LCMRglc are more pronounced in cortical association areas than in other regions and emerge coincident with the appearance of sex differences in complex social behavior, suggesting a critical role of sex differences in the development of metabolic differences in LCMRglc and behavior. Supported by the Dana Foundation, the Veterans Administration, and the NINDS.

**T94.6**
THE ROLE OF CELLULAR ACTIVITY IN THE DEVELOPING CEREBRAL CORTEX. G.Maguire, and D.J. Price. Department of Physiology, University Medical School, Edinburgh EH8 9AG, UK.

An in vivo organotypic culture system was used to examine the development of the thalamocortical system. In culture embryonic day 15 thalamic innervates postnatal day 6 cortical explants and axons terminate within layer 4, as in vivo. Our hypothesis is that the release of neurotransmitters contributes to the determination of cell fate and target selection within this co-culture system. Neurotransmitter release may require or induce fluctuations in activity.

We either added tertrotodin (TXX), a sodium channel blocker, or increased potassium levels (to depolarize cells) in the developing co-culture system. TXX (10 M) resulted in increased thalamic outgrowth and eliminated the recognition of layer 4 by thalamic axons. KC1 (5 x 10 M) resulted in excessive thalamic and cortical outgrowth and abolished target recognition. TXX and KC1-treated co-cultures were sectioned and Nissl stained to reveal effects on cell viability. Preliminary results indicate that TXX enhances cortical cell viability, perhaps by reducing glutamate induced cell death in these co-cultures. These experiments support a role for activity in the determination of cell fate and target recognition in the cortex. We are currently investigating whether neurotransmitters are involved in these processes, by adding specific receptor antagonists to the co-culture system.
T89.1

INTRINSIC INTERLAMINAR CONNECTIONS IN AREA V2 OF MACAQUE MONKEYS ARE ALTERED AFTER MONOCULAR DEPRIVATION. L. Dippel, D. Aaij, L. S. Johnson, and M. H. Tarr. 22, Dep. of Psychology, Vanderbilt University, Nashville, TN 37240. Work supported by NIH grant EY1366.

We studied the organization of intrinsic laminar connections in V2 of macaque monkeys monocularly deprived from birth, either surgically removed of the lens (aphakic group) or by creating a black contact lens (exclusion group). Connections of cortical layers 1-2 groups and in contralateral and non-deprived monkeys were measured by labeling 100-200μm injections of the Fluoro retrograde tracer into visual cortex and slicing the brain. Within 2 mm thick cortical slices incubated 3-4 h in a chamber with oxygenated Ringer's solution and then cut into 50μm sections. Sections were stained for fluorogold. The section pairs were then collected and sectioned in lateral borders. All injections produced normal retinal bands 200-300μm in diameter of dense label within columns extending across cortical layers. Observations of transferred sections depended on the laminar location of the injection. Injections in supragranular layers produced the densest label in layers 2/3 and 5/6 and this label was often lateral to the injection site. Label in layers 4 and 6 was always more confined. Injections in infragranular layers led to dense widespread label in layers 5/6 and more confined label in layers 2/3. Monocular deprivation in monkeys exhibited differences from normal in density and lateral extent of horizontal connections. In contrast with controls, horizontal connections were denser in occluded and spared in aphakic monkeys. They were unusually widespread in occluded monkeys, extending laterally up to 4mm, and often unevenly distributed, forming zones of dense and sparse label. Cytologically, monocular deprivation in aphakic animals was restricted in contrast with controls (approx. 0.5 and 1mm from the injection site, respectively). These data show that deprivation effects can be traced across functional columns, the geniculostriate system. (Supported by EY-0973; RR-00165 and EY-0266).

T89.2


In the cat, the primary visual cortex (area 17 or V1) receives its input from the lateral geniculate nucleus (LGN) through two pathways: the magnocellular and parvocellular streams. The magnocellular stream carries visual information related to motion and the parvocellular stream carries visual information related to form and color. The two streams converge in V1 through horizontal connections, which play a significant role in the processing of visual information. The study of these connections is crucial for understanding the mechanisms underlying binocular vision, stereopsis, and motion perception.

The study by Council et al. investigated the directional sensitivity and fusion limits of vertical association pathways in the cat. They found that the vertical association pathways are directional and that their fusion limits are related to the eccentricity of the visual field. These findings provide insight into the mechanisms of binocular vision and the role of horizontal connections in visual processing.
VISUALLY EVOKED POTENTIAL (VEP) ASSESSMENTS OF VISUAL FUNCTION IN A MONKEY MODEL OF TREATMENTS FOR HUMAN UNILATERAL INFANTILE CATARACTS. A.D. Ross, J.L. Brown, L.C. Stevens and R.G. Boothe*. Division of Neurobiology and Vision, Yerkes Research Center, Emory University, Atlanta, GA 30322.

The purpose of this study was to analyze treatment strategies for children with unilateral infantile cataracts. Infant rhesus monkeys received a lensectomy within 10 days of birth to mimic the surgical treatment that is typically given to human infants. Thirteen infant monkeys were placed into two groups. The first (AFP-NP) were treated with a lens implant that obscured the aphakic eye to a far point and the fellow eye to a near point. The rationale for this treatment was that it would improve binocular vision for far targets and the fellow eye when viewing near targets. Targets at mid distances would be expected to provide some binocular stimulation. The second group (AFP-NP) were anesthetized to remove the aphakic eye and the fellow eye to a near point, and an occluder contact lens on the fellow eye for up to 90% of the time. The rationale for this group was to use part time occlusion to force usage of the aphakic eye. There were no significant differences in visual acuity between the two groups at 3 years of age. Behavioral assessments with preferential looking methods during the rearing period indicated good acuity development in all eyes. However, VEP assessments at 3 years showed that the acuity of the aphakic eyes of the AFP-NP group had deteriorated substantially while acuities in the AFP-NP group were relatively stable. All animals exhibited directional motion asymmetry which is a marker for impaired binocular function, although strabismus was not as prominent in the AFP-NP group. It is concluded that selective treatment methods are somewhat better at promoting binocular function, but part time occlusion methods are better at maintaining spatial vision. Supported by EY05975 and RR01615.

DEVELOPMENT OF CAMP-LINKE D METABOTIC GLUTAMATE RECEPTOR (mGluR2/3) AND DARK RARING INFLUENCE ON mGluR5 (1, 2A AND 5) IN THE CAT VISUAL CORTEX. C. Romans, L.G. Olyphant, J.B. Davis, D. Declain and W. Drey. Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven CT 06520-8061; Department of Ophthalmology and Visual Science, Washington University School of Medicine, St. Louis MO 63110.

Metabotropic glutamate receptors (mGluRs) are involved in NMDA-dependent and NMDA-independent synaptic plasticity. These receptors may also participate in postnatal, development-dependent plasticity in the visual cortex. mGluRs are coupled to various signal transduction pathways. In this study, we examined the developmental profile of CAMP-linked mGluR2/3. We also examined effects of dark-rearing on mGluR5 and PI-linked mGluR2/3. We found that the laminar distribution of mGluR2/3 changes with age. The change is different from either mGluR1 or 5. Besides laminar distribution, the quantity of mGluR2/3 also changes during postnatal development. In contrast to mGluR1 and 5, mGluR2/3 increases during the early postnatal stage. Dark-rearing increases the quantity of mGluR5 at the peak of the critical period for monocular deprivation, however, it has little effect on the quantity of mGluR1. As in the case of mGluR5, dark-rearing affects the laminar distribution of mGluR2/3. These results suggest that these receptors are probably involved in different developmental processes of the visual cortex. mGluR2/3 and mGluR5 are involved in sensory-dependent events, but mGluR1 is not. Supported by ROI EY 00053 and HSFP05.

PROBING THE "PLASTICITY GATE" IN VISUAL CORTEX USING PULSE-PULSE STIMULATION. P. Hare, A. Kirkwood, M.A. Parada* and M.E. Baum. Dept of Neuroscience and HHMI, Brown University, Providence, RI 02912.

Previous work in our lab has shown that high frequency stimulation of a site in the middle of the cortex (corresponding to layer IV), but not of white matter (WM), results in LTD of layer III field potentials (FPs) in visual cortical slices from adult rats. Since we know that the hippocampal-LTP is blocked by within a deep to layer IV normally acts as a "plasticity gate": a filter that constrains the types of activity patterns that can gain access to the modified synapses in layer III. In this study we reasoned that paired-pulse stimulation to the layer IV would be expected to block the different patterns of synaptic activation that result from high-frequency stimulation of layer IV. We included layer IV as part of the same area of cortex that was used in layer III as different sites were stimulated. In each experiment, paired-pulse stimulation was applied to the WM while systematically varying stimulus strength and inter-stimulus intervals. The magnitude of the synaptic responses to the pulse trains were compared with those of the control stimulation. The data show that highfrequency stimulation of layer IV is able to block the LTD that is induced by stimulation of layer IV. However, at stimulation intensities yielding a FP ≥ 50% of the maximum, the amplitude of the LTD is still significantly reduced compared with control stimulation. The data show that high-frequency stimulation of layer IV is able to block the LTD that is induced by stimulation of layer IV.
VOLVEMENT OF PROTEASES IN OCULAR DOMINANCE PLASTICITY IN KITTEN VISUAL CORTEX C.B. Grünwald* and C.M. Müller, Max Planck Institute for Developmental Biology, Tübingen, Germany
Ocular dominance stripe segregation during visual cortical development relies on selective growth and retraction of the initially overlapping ganglion-corticopialfferents from either eye. As suggested by recent in vitro studies, the molecular mechanisms underlying such axonal remodeling may include the action of proteases, such as the plasminogen-activator/plasmin system (Pitman et al., J Neurochem 70:621-626,1998). We have investigated the role of proteases in ocular dominance plasticity by chronically infusing serum-protease inhibitors into the visual cortex of kittens by means of multicontralateral scalp perforations. We performed monocellular deprivation (MD) or reverse occlusion (RO) for 7 days. Thereafter we electrophysiologically assessed ocular dominance (OD) distribution, orientation selectivity, and response strength. Leupeptin (100μM), a broad spectrum inhibitor, not only failed to influence the OD shift after MD. Further, the reversal of OD after RO was significantly retarded by leupeptin infusion (10.9% reversal index compared to 76% in controls). Orientation selectivity and response strength were unimpaired. Since leupeptin exhibits strong inhibitory activity against plasminogen, but only weak affinity for thrombin, we tested the highly potent thrombin inhibitor hirudin (50μM) and SDS 217-766 (100μM; gift of Sandor LTD, Switzerland) in additional MD experiments. While hirudin failed to influence OD plasticity, the low molecular weight inhibitor SDS 217-766 attenuated the OD shift towards the experienced hand (21.7% in controls). The results indicate a role for leupeptin-sensitive proteases in progressive plasticity after RO, most likely mediated by the plasminogen-activator/plasmin system, known to participate in axon growth. Here, leupeptin changes like synapse elimination during MD. Supported by the BMBF 0316902

DEVELOPMENT OF CORTICAL BINOCULAR DISPARITY TUNING AND CORTICOGENICULATE FEEDBACK A. Grünwald* and C. Grossberg, Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215
The rapid processing of binocular disparity information requires highly tuned disparity-selective neural networks, yet at birth infants only show a coarse level of sensitivity. A model shows how complex cells can develop fine disparity tuning starting from coarse tuning. Competition across cortical complex cell columns leads to an exact scalar (1D) model, while tuning strength for dimensional dissociation is allowed to learn the pattern of activities that feed into the complex cell. Antagonistic rebound responses at the retina ensure that whenever learning occurs for one type of edge contrast at the complex cell stage, learning also occurs for the complementary edge contrast. In this way, complex cells develop to be insensitive to the polarity of contrast. At the same time, complex cells learn to fuse only stimuli where both eyes have the same polarity of edge. Whenever a complex cell emerges as the winner, a top-down matching, or confirmation, signal is sent to the LGN. This confirmation signal stabilizes the learning process. When the confirmation signal matches the LGN activity patterns, then the matched LGN activities are amplified. A mismatch between the confirmation signal pattern and the LGN pattern leads to a reduction of LGN activities so that a new winner is picked at the complex cell stage. Feedback signals are disparity-tuned, because, whenever a winner is picked at the complex cell stage, learning also occurs in the feedback pathway. Thus the model explains the importance of cortico-geniculate pathways for the self-organization of disparity tuning.

Correlation-Based Learning Model of Joint Orientation and Ocular Dominance Column Formations. E. Ergin* and K.D. Miller, W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco CA 94143
Previously, we have shown that correlation-based competition between left- and right eye inputs can lead to development of ocular dominance (OD) columns, while that between ON- and OFF-center inputs can lead to simple cell receptive fields (RFs) and orientation (OR) columns. Here we develop a similar model involving four input types, ON- and OFF-center inputs from left and right eyes. We assume time- invariant input correlations. We have determined the conditions for concurrent development of ocular dominance and orientation columns with preferred orientation varying continuously across OD boundaries. We are studying the relationships that then develop between the two column systems. We assume symmetry between left and right and between ON and OFF. This leaves four independent correlation structures: those between input cells of same or opposite polarity each within the composite center type. By a linear transform, the development equations decompose into four independent equations describing development of receptive fields and of maps of four synapses with two combinations: (1) the sum of all four weight types (SUM); (2) left-eye minus right-eye weight (OD); (3) ON-center minus OFF-center strength (OR-); (4) ON-center minus OFF-center from left eye, plus OFF-center minus ON-center from right eye (OR-). Each weight combination develops independently under a distinct correlation function derived as a linear combination of the original four correlation functions. We call these functions CRON, CROS, COR- and CRC-. Jointly, the correlated orientation preference develops in a large regime where (1) CRON is positive over the arbor radius (radius of LGN cells converging onto a cortical cell), favoring monocular RFs; (2) the Fourier transform of CRON has a single peak at a wavelength responding to 0.6-1.0 arbor diameter, favoring oriented RFs; (3) the principal eigenvalues associated with CROS and CRC- are of comparable size and larger than the eigenvalues associated with the other two functions. These other two functions are otherwise largely unrestricted in structure.
Supported by NIH grants NS07987 and EB11081-01.
NON-INVASIVE DOPAMINE DETERMINATION BY REVERSED PHASE HPLC IN THE MEDIUM OF FETAL MESCENPHALIC CULTURES: A TOOL TO PRIORITIZE THE CAPACITY FOR NEUROTRANSPLANTATION.

Y. H. Chang* and E. C. Zhou, Program in Medical Neurobiology and Dept. of Anatomy, Indiana Univ. School of Med., Indianapolis, IN 46202

Epidural grafting (EGF) secretes and supports multipotent neurons from either adult or fetal brains (Reynolds, et al. ’92), and fetal sympathetic ganglia (Silani, et al. ’94). To simplify previous labors and procedures of dissociation and obtain a greater number of large size neurons, we adopted a new non-passage method of procuring EGF. EGF was extracted after ultrafiltration by an analytical kit (Chromsystems, No. 5000) and probes were determined with a reversed phase HPLC using electrochemical detection (ESA, Mod. 5011).

The mean dopamine concentration in medium derived from one culture increased from 21 ± 11 ng at day 4 to 58 ± 43 pg at day 12 and decreased to 39 ± 30 pg at day 16 (N=24 each). In all cultures devoid of dopamine after 4 and 8 DIV (12.5%) levels remained below detectability at 12 and 16 DIV. There was a substantial variation in dopamine levels of medium derived from cultures of different embryos ranging from 82 to 282 pg dopamine. Statistically higher dopamine levels were detected in medium derived from cultures of the rostral part of the mesencephalon compared to that from cultures of the caudal area. After 12 and 16 days in vitro, 78% and 81% of dopamine was localized in 50% of the cultures assessed. These results show the feasibility of non-invasive individual characterisation of NT2-N cell cultures of human fetal mesencephalon prior to transplantation.

SUPPORTED BY SRFNo. 31-36243-92 and by NBW No. 93.0349.

T66.4
HUMAN NEUROBLASTOMA NT2-N CELLS (NT2) IMPLANTED INTO MURINE SPINAL CORD MATURE AND INTEGRATE ALONG THE HOST ARCHITECTURE INDEPENDENT OF HOST AGE OR LOCATION


NT2 cells, derived from a human teratocarcinoma, were differentiated into neuro-like cells (NT2) with retinoic acid and implanted into the spinal cords of nude mice to investigate the influence of host factors on graft integration and survival. NT2 cells were implanted in the dorsal, caudal or central cord in either midline or lateral locations. Post-implantation survival was assessed by tyrosine hydroxylase (TH) immunostained serial sections of the grafted brains between 7 to 27 days post transplantation.

The survival of transplant animals showed large, viable grafts containing TH-immunoreactive (TH) cells. The density of TH+ neurons in these human fetal xenografts was 194±4/404 TH+ cells/mm² (N=26). No significant difference was detected when TH+ cell density of grafts derived from human foetuses EA 43 to 56 days post conception was compared to grafts from human foetuses EA 56 - 72 days post conception. This shows that ventral mesencephalic tissue from foetuses older than 8 weeks can be successfully cultured and transplanted when using the free floating roller tube technique. This process provides the feasibility of in vitro maintenance of fetal human neural tissue.

SUPPORTED BY SRF No. 31-36243-92 and by NBW No. 93.0349.

T66.5

The immortalized mesencephalic cell line, IRBAN, has several neuronal and dopaminergic properties that make it a candidate for neurotransplantation in Parkinson's Disease. In order to further characterize the cells, we stained IRBAN cells with antibodies directed against the neuronal marker, neuron specific enolase (NSE), the astrocytic marker, glial fibrillary acidic protein (GFAP) and a marker of neuronal progenitor cells, nestin. For positive controls, cultures of dissociated E15 dopaminergic neurons were analyzed by nestin and GFAP. The size of these cells was smaller than that of nestin-negative cells. Cells inside and outside of neureps were proliferative as indicated by PCNA immunostaining. Immunoreactive S-HT, GABA, 5-HT1A receptor, MAP2 were observed in the cells immunohistochemically. Cells with glial morphology stained positive with antibodies against GFAP and S-100. NESTIN results suggest that EGF-responsive non-neuronal progenators possess nestin and continuously proliferate. They can give progeny of both neuron and glia even for 3 to 10 months in culture.

T66.6
GENETIC METHODS FOR INCREASING THE VIABILITY OF FETAL MESCENPHALIC NEURONS IN VITRO AND IN GRANTS

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In an attempt to increase the number of viable dopaminergic cells following in vitro implantation of fetal ventral mesencephalic (VM) cells, different genetic techniques are being explored. First, we have constructed both recombinant adeno-associated virus (AAV) vectors and herpes simplex virus (HSV) amplicons containing the human beta-2 gene. These constructs were delivered to their appropriate helper competes particles and current are being used to directly infect dissociated E12-14 VM cells prior to transplantation into unilaterally 6-OHDA lesioned rats. Graft viability and functional integration are evaluated by measuring the reduction in amphetamine-induced rotation and by characterizing immunohistochemically the cellular composition and gene expression within the graft. Second, in order to stabilize vector-mediated gene expression, we have been performing transgenic mice expressing the absence of the AAV rep gene. Other methods of direct gene transfer into fetal neurons are being investigated, including transplacental delivery of plasmids.

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Benzodiazepines, barbiturates, volatile anesthetics, and alcohol all act on GABA receptors. Because such GABA mimetics are widely used to treat anxiety, seizures, and movement disorders, direct delivery of GABA to such sites may be experimentally and therapeutically useful. GABA, a neurotransmitter, cannot cross the blood-brain barrier, but transplanted engineered cells offer a method for the in situ delivery of GABA in experimental models of movement and seizure disorders. With this possibility in mind, we have used retroviral vectors containing GABA α1 or δ receptors to transfect neural progenitor cells. We have studied several such lines, including a number from AIT-20 cells, a tumor of mouse anterior pituitary. These engineered cells produce appropriate GADs, GAD mRNAs, and GABA. Unstimulated cells release accumulated GABA at a basal level, as determined by HPLC analysis. Treatment with 8-BrcAMP stimulates the GABA release, but depolarization with 50 mM KCl does not. Our data suggest that, in engineered AIT-20 cells, GABA accumulates in large, dense-core vesicles that contain ACTH.


We previously reported that conditionally immortalized neuronal progenitors and primary neural progenitor cells isolated from the E14 rat telencephalon survive upon transplantation into the embryonic rat brain and organize into clusters of cells that progressively lose amacrine markers characteristic of immature cells (Caneau et al. Dev. Brain Res. 1994). In this study we adopted the same experimental procedure to study the in vivo behavior of GFRα-responsive human stem cells lines isolated from the dentate zone of 10 week old ferrets. Human donor cells were subcultured for up to 2 years and were exposed to 1µM BrdU, 6 days prior to transplantation. On the day of the transplant, cells were loaded with Dil. The animals were sacrificed on postnatal day 1. Following virename and cryostat sectioning, cells were found widely dispersed and integrated into the host brain and processes were often visible as judged by the Dil signal. The chromatin inside the nucleus of the Dil labeled cells was also distinguishable after Hoechst staining due to its more dispersed nature and lack of prominent chromatid spots. Despite the heterogeneous nature of the donor cells, no cluster segregation of the transplanted cells was ever observed following transplantation. Clear cases of BrdU and Dil double labeled cells were visible. These results indicate that fetal transplant may represent a suitable way to test for the stability and differentiation potential of non-transformed human CNS stem cells partly supported by the Alzheimer's Association P5G-9057 to E.C. and by funds of the Italian Ministry of Health to A.L.V.}


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Neural transplantation following brain injury is a potentially promising technique for promoting functional recovery. The present study examined the possibility of transplanting EGF-responsive neural stem cells into injured tissue and analyzed the graft efficacy in supporting behavioral recovery. Progenitor cells isolated from E17 mouse subventricular zone were mechanically dissociated and plated in serum free medium containing EGF (Reynolds and Weiss, Science 255:1707, 1992). After 5-6 DIV, spheres of mitotically active cells growing in suspension were harvested labeled with Dil (Molecular Probes Inc.) and utilized for transplantation into the caudate/putamen of ischemic rats. Focal ischemia was induced in S-O rats by a suture model to produce 'infarction'. The animals were sacrificed 2 weeks after ischemia. Viable cells were clearly detected 30 days following transplantation, the latest time examined. Although most cells remained near the site of infarction, there was some migration of cells away from the site. We are currently examining whether cell grafting ameliorates behavioral deficits due to the stroke. Nevertheless, the survival of progenitor cell grafts in ischemic brain suggests that cell grafting may provide a means of intervening therapeutically after stroke.


R.E.C.-M17, a human neuroblastoma cell line, and NT-2, a human teratocarcinoma cell line with the tyrosine hydroxylase (TH) gene transferred (kindly provided by Dr. R. Flobbins), were stereotactically grafted in four different regions of the rat brain: i) dorsal thalamus, ii) substantia nigra, iii) cerebellum, striatum and amygdala. Rats (n=55) received injections of suspended M17 or NT-2 cells in HIBS (1.8×10^6 cells). Controls received an injection of vehicle only. At 1, 4 and 8 weeks after transplantation, rats were anesthetized and perfused with 10% formaldehyd. The brains were sectioned and immunostained for TH and GFAP. The grafted M17 and NT-2 cells survived up to two months post-transplantation. NT-2 cells never appeared to revert to a neostriatal state. By contrast, the M17 grafted cells generated tumors within 15 days after transplantation in 30% of the grafted rats, generally affecting the striatum and amygdala grafts. The cells stained positively for TH in viva but failed to stain in vivo. At 1 week post-grafting the M17 and NT-2 cells were rounded. At 4 and 8 weeks post-grafting most M17 cells were polygonal and extended neurite-like processes, while NT-2 cells were round or fusiform without processes. M17 cell grafts were strongly vascularized by invading host brain capillaries.

Our results show that both cell lines can survive for at least 2 months after intracranial implantation, but they differ with respect to their pathogenicity. While the M17 cell line generated tumorigenic potential, the NT-2 cell line did not. Supported by MEC-P 86-01066 and DGICYT.
EMBRYONIC STEM CELLS TRANSPLANTED TO THE ADULT BRAIN: TYROSINE HYDROXYLASE (TH) POSITIVE NEURONS DEVELOP SPONTANEOUSLY AND BY TRANSFERENCE WITH HUMAN TH GENE. T.W. Deacon,1 J. Dismore, 18., W. Gardiner, and O. Jackson.1 McLean Hospital/Harvard Medical School, Belmont, MA 02128; 2) Diacrin, Inc., Charleston MA 02129

Embryonic stem (ES) cells derived from mouse blastocyst and maintained in culture with leukemia inhibitory factor (LIF) were transplanted into the lateral ventricles, striatum, cerebellum and neocortex of adult mice. Before transplantation they were subjected to three different treatments: a) retinoic acid (RA) treatment, b) transfection with a plasmid containing the human tyrosine hydroxylase (TH) gene, or c) left untreated. Rat host cells were immunosuppressed with Cyclosporin A; mouse hosts were not immunosuppressed. Untreated and RA treated cells produced grafts with variable neurite outgrowth. TH-positive neurons were produced in all three groups. However, we found that cells differentiated and were capable of producing exogenous gene products in vivo, allowing for the expression of candidate genes that might not only enhance differentiation and engraftment of these cells but also permit host SC function following insult through the regeneration of endogenous cells and circuitry. (Supported in part by grants from the APA and PVA).


Lesion-induced host factors, particularly trophic support and denervation, dramatically enhance cell survival in micrografts transplanted to adult rat hippocampus at 4 days post-Kainic acid (KA) lesion [Shetty and Turner, Neuroscience]. We hypothesize that restoration of hippocampal throughput in the KA model critically depends on the establishment of specific graft-host connections. Large supragranular grafts of E19 hippocampal cells were prelabeled with 5-bromo-2′-deoxyuridine, and were transplanted at 4 days post-KA lesion. We have measured the formation of effector connections after 4 weeks of survival using FluoroRuby, DiI tracing and serial section analysis. The effect of grafts on aberrant sprouting of mossy fibers into the dentate supragranular layer (DSL) was also quantified in Timm’s stained sections.

Many infants in KA-lesioned rats demonstrated reinnervation of the CA3 cell layer established commissural connections with the contralateral hippocampus (MeanSEM=56±328, 87). However, such connections did not occur with ectopic grafts in untreated, untreated animals. Neurons within all grafts made connections with the medial septal nucleus (451±17, 76). Host mossy fiber ingrowth into the graft area was denervated for grafts located near the denervated CA3 cell layer but not other grafts within hippocampus. Grafts of donor hippocampal cells expressing different domains of the DSL (measured as both width and area per length) was significantly reduced in animals with grafts near the CA3 cell layer (n=5, p<0.01, 58% reduction) compared to animals with ectopic transplants (n=9). These results clearly show that fetal hippocampal neurons both reconstitute the damaged circuitry following KA lesions by establishing specific, top-to-point connections and significantly reduce the development of post-KA abnormal circuitry in hippocampus. Supported by ROI NS29482-01 and VAMC.
**T97.1**


Glia fibrillary acidic protein (GFAP) is a astrocyte-specific intermediate filament protein which is used as an index of reactive gliosis and neurodegeneration. GFAP increase in response to brain injury and normal aging, and its expression can be manipulated by altering circulating adrenal and gonadal steroids. Gonadal steroids decrease in women at the age of menopause, and testoterone concentrations are often reduced in older men. One hypothesis is that lower levels of gonadal steroids might render the brain more susceptible to neurodegeneration. The purpose of this study is to examine the effects of gonadal steroid manipulation on GFAP in the rat brain during aging and the process is regulated. Two groups (3, 12, or 24 weeks) of male, Fisher 344 rats were castrated and given hippocampal lesions. Castrated animals received either testosterone implants or blank implants, resulting in marked differences in the concentration of circulating testosterone. In this preliminary study, GFAP immunoreactivity in the cerebellum (a region not affected by the lesion) was measured by ELISA and compared between age and hormone treatment groups. The results indicated that 3 and 12 mo intact and castrated animals had lower GFAP content than their respective 24 mo groups. Testosterone implants in castrated rats reduced this age-related increase in GFAP immunoactivity. These data show that the exogenous testosterone suppressed the age-related increase in GFAP in the cerebellum, a brain region not generally associated with steroid hormone sensitivity. These data support the hypothesis that changes in gonadal steroid hormones during aging might render the brain more susceptible to neurodegeneration. Also, these data suggest that hormone replacement therapy might have value in neurodegenerative disease intervention. This study was funded by an AFAR research grant to J.R.D.

**T97.2**

**AGE RELATED INCREASE IN TGF-B1 mRNA IN RAT HIPPOCAMPUS IS LOCALIZED TO ACTIVATED MICROGLIA: N.R. Bishko*, C.E. Finch and T.E. Morgan, Dept. of Neurobiology and Neurosurgery, University of Southern Calif., Los Angeles CA 90089-0191.**

Increased TGF-B1 expression occurs in adult brain in response to injury and disease and may mediate neurotrophic effects of gli. Previously, we showed by RNA blot hybridization that TGF-B1 mRNA was up regulated in rat and human brain with advanced age. We also showed that TGF-B1 was regulated after lesioning of rat brain and was localized to activated microglia following both deafferenting and neurotensin lesions. Several reports indicated that microglial numbers increased during aging and that microglia in the aged brain exhibited an activated morphology (shrunken, thickened processes and increased expression of complement receptor 3 and TNF-a). These data suggested that the age-related increase in TGF-B1 mRNA could be due to the increased number of microglia or to increased expression per cell. Therefore, TGF-B1 mRNA was quantified in the hippocampus and cortex on a per cell basis in young (7 mo) and old (24 mo) F344 male rats following in situ hybridization with a [35S]-labeled cDNA probe. TGF-B1 grain density was measured in young and old rats. The results indicated that increased TGF-B1 mRNA prevalence is a marker of activated microglia in the aged hippocampus and cortex. If increased TGF-B1 expression is secreted from activated microglia, this peptide may contribute to the neurotrophic activity of microglia in the brain during aging. Supported by AG-07909 (CEF) and AFAR (TEM).

**T97.3**

**TRANSFORMING GROWTH FACTOR (TGF)-B1 AND THE MICROGLIAL RESPONSE TO AGING: T.E. Morgan, J. Rozovsky, T. Hogrel, and C.F. Finch, Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.**

Unlike microglia in young adult mammalian brain, microglia in aged brains appear to be in an activated state that we hypothesize may contribute to the age-related susceptibility to neurodegeneration through altered responses to TGF-B1. TGF-B1 is a pleiotrophic peptide that increases during the brain’s response to neurodegeneration and aging. In cultured microglia from neonatal rats, TGF-B1 effectively suppresses activation, proliferation, and major histocompatibility class II expression. These studies examine the effects of TGF-B1 on cultured microglia from aged rat brains. As observed in vivo, cultured microglia from 24 month-old rat brain (“aged”) are morphologically distinct from their “young” (3 month old) counterparts and appear to be in an activated state. The mitotic index (MI), calculated from “H-thymidine labeling, of “aged” cultured microglia is 4 times higher than the MI of “young” cultured microglia. TGF-B1 (1 ng/ml, 24 hours) treatment does not inhibit the increased proliferation of “aged” cultured microglia. Furthermore, treatment (1 ng/ml, 24 hours) failed to inhibit the lipopolysaccharide (LPS)-induced formation of reactive nitrogen intermediates (nitrite concentration in medium) in “aged” cultured microglia. They are less responsive to the deactivating effects of TGF-B1, which may be a factor in age-related diseases, such as Alzheimer’s disease. Supported by AG-07909 (CEF) and AFAR (TEM).

**T97.4**

**FOCAL SITES OF DEMYELINATION AND REMYELINATION (MICROPLAQUES) IN PERIPHERAL NERVES OF AGED CATS: C. Bentzon, J.K. Engstand, F.R. Morales and M.H. Chase, Department of Physiology, Department of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.**

Previous morphological studies in our laboratory have demonstrated that segmental demyelination and remyelination are present in the peripheral nerves of old cats (Bertolotto C. et al. Soc. Neurosci. Abstr. Vol. 20, part 2, p. 1710, 1994). In the process of examining myelin alterations during aging, we found that demyelination and remyelination exhibited a consistent pattern along the fibers of peripheral nerves of old cats, which is the subject of this abstract.

Two adult cats (1 to 3 years old) and three old cats (17 to 19 years-old) were used in this study. These animals were given a lethal dose of sodium pentobarbital and perfused with a saline solution and a fixative (2% paraformaldehyde and 2% glutaraldehyde). The head was mounted in 0.1 M phosphate buffer pH 7.4. Tissue preparations of the hind limb nerves were stained with 1% osmium. The fibers were mounted in pure glycol methacrylate and examined with a light microscope.

Teased myelinated fibers from the adult cats exhibited a smooth myelin surface with intact nodes of Ranvier. The teased profiles of each individual fiber were of similar thickness and length. The teased fiber preparations from the old cats exhibited regions that contained clusters of two or more adjacent fibers where demyelination or remyelination occurred. These regions were delineated by apparently normal myelin. We refer to these regions, that appeared as small patches of abnormal myelin, as "microplaques." These focal abnormalities in adjacent myelinated fibers in the peripheral nerves, to our knowledge, have never been described before in aging animals or in peripheral neuroopathies. However, we believe they were present in previous studies of others because published microphotographs exhibit the same phenomena that we have described in the present report. The presence of microplaques suggests that, during aging, there is a focal process that affects clusters of Schwann cells which are located in close proximity to each other. Supported by USPHS Grant AG 04307.

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**797.5**

**DISTRIBUTION OF APOLIPROTEIN E PLAQUES AND GLOBOSIS IN THE AGING RHESUS MACAQUE TEMPORAL CORTEX.** S.G. Kohama* and H.F. Urbanik. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, Oregon, 97006.

Expression of the astrocyte marker, glial fibrillary acidic protein (GFAP) increases during aging and neurodegeneration. Similarly, the expression of apolipoprotein E (ApoE) increases with age in nonhuman primates in association with amyloid deposits. Because of the potential role of both of these markers in Alzheimer’s Disease, the relative distributions of GFAP and ApoE were examined in the temporal cortex of the aging rhesus macaque (Macaca mulatta).

The distribution of GFAP in the temporal cortex of young and middle-age adult monkeys was similar, with high levels seen in the molecular layers of the dentate granule cells, Ammon’s horn and glial limitans. In some older monkeys (>25 years), hypertrophic astrocytes could be visualized in the hippocampal pyramidal and deeper layers of the temporal cortex.

ApoE expression was only seen in the older monkeys. Although a few plaques were seen scattered in the hippocampus of several of these animals, the majority of plaques were seen in the temporal cortex. Thus the more generalized distribution pattern of reactive astrocytes overlaps with that of the ApoE plaques, suggesting that a causal relationship may exist between astroglialosis and ApoE deposition.

Grant Support: Alzheimer’s Assn. P30-94-123 and NIH HD-29186

**797.7**

**AGE-ASSOCIATED CHANGES IN CNS TRANSCRIPTION FACTOR ACTIVITY.** T. Toliver*, J. Papacostas and R. Perez-Polo. Dept. of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, Texas 77555-0652.

Age-related cognitive deficits are usually associated with a loss of cholinergic function. Transcription factors may play an integral role in regulating cholinergic homeostatic gene expression. In the present study, we tested the hypothesis that transcription factor nuclear factor xB (NFxB) activity is altered in the basal forebrain and hippocampus of aged rats. Nuclear extracts prepared from basal forebrains and hippocampi of 3 and 30-month old Fischer-344 Brown Norway rats were used to measure NFxB activity by electrophoretic mobility shift assay. Basal levels of NFxB binding to cognate DNA consensus sequences were significantly higher in hippocampi (p<0.01) and basal forebrains (p<0.05) of aged rats. Basal NFxB activity was also measured and showed no significant difference between age groups. NFxB activity in cerebral and frontal cortex was also measured and there appear to be no significant differences between age groups. The data demonstrate increased basal levels of NFxB activity in the basal forebrain and hippocampus of the aged rat. The regional differences suggest a possible relationship between altered NFxB activity and the decreased neurotrophin action and cholinergic function associated with aging. Supported in part by NINDS NS18708. This is publication #13A and is supported by USBHR grant P81AG10514 awarded by NIA.

**797.9**

**BDNF AND SOMATOSTATIN GENE EXPRESSION IN THE PRIMATE BRAIN: DECREASED LEVELS OF mRNA DURING AGING.** M. Havia* and K. Shimizu. Department of Cellular and Molecular Biology, Primate Research Institute of Kyoto University. Inuyama, Aichi 484, Japan.

BDNF (Brain Derived Neurotrophic Factor) is one of the neuritogenic molecules for the various neurons in the vertebrate central nervous system. A recent observation was the marked decrease in BDNF gene expression in the hippocampus in Alzheimer’s disease. Furthermore, in the rat cerebral cortex, BDNF was involved to enhance the level of mRNA of the neuromodulator somatostatin, suggesting that BDNF may be a regulatory molecule for the expression of the somatostatin gene. In the present study, using the northern blot analysis, we investigated BDNF and somatostatin mRNA expression in the central nervous system of the macaque monkey (Macaca fascicularis). During the aging process, BDNF and somatostatin mRNA (1.6 and 4.0 kb transcripts for BDNF, 6.65 kb transcript for somatostatin) were detected in various cerebral subdivisions (the frontal cortex, the temporal cortex, the motor cortex, the somatosensory cortex and the visual cortex) and the hippocampus. During the aging process (2 years, 10 years and >30 years), the levels of BDNF and somatostatin mRNA significantly decreased in the cerebral subdivisions and the hippocampus. These findings suggest that the decrease in the gene expression of BDNF may cause the levels of somatostatin mRNA to decline in the primate brain during the aging process. Supported by Grant-in-Aid (04268103) from the Ministry of Education, Science and Culture, Japan.

**797.6**

**ALTERATION OF MULTIPLE GENE EXPRESSION IN INDIVIDUAL HIPPOCAMPAL CA1 NEURONS AS A FUNCTION OF AGING.** Y.Kato*, T. Akagi and J. Uehara. Dept. of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Aging is recognized as a multifactorial event. The study of the interactions between these factors is essential to understand the fundamental mechanisms of the aging process. Using the antisense RNA amplification (aRNA) technique, we would like to simultaneously analyze multiple gene expression in individual cells in live acutely cultured slices of the hippocampal CA1 field. 3-month-old, 12-month-old and 24-month-old Fischer 344 and Brown Norway F1 hybrid rats were used in the present study. We found that the mRNA levels for individual subunits of the glutamate receptor family were altered; specifically GluR2, GluR5 and GluR7. mRNAs decreased as a fraction of total composition of these receptor subunits as a function of age while GluR1, GluR3, GluR4 and GluR6 mRNAs had no significant compositional changes. Superoxide dismutase (SOD) mRNA also showed age-related changes, and interestingly the type II glucocorticoid receptor mRNA had higher expression in some but not all neurons from aged hippocampal slices. Presuming that protein levels parallel mRNA levels, then it is reasonable to postulate that coordinate changes in gene expression are reflective of functional significance.

**797.8**


Age-dependent spatial learning impairments have been related to a decline in hippocampal plasticity. It has been shown that highly polysynaptically connected neuronal cell adhesion molecule (PSA-NCAM) are highly expressed during adulthood within regions associated with ongoing neurogenesis such as the hippocampus. Furthermore, NCAM deficient mice lack PSA-NCAM and show deficits in spatial learning when tested in the Morris water maze. The aim of the present study was to examine the effect of aging on the expression PSA-NCAM within the hippocampus and other various brain regions using immunohistochemistry. In addition to investigate whether age-dependent changes in expression PSA-NCAM were accentuated in aged rats with learning impairment, animals were in a first step assessed for their memory capability using a Morris water maze. Seven-month-old and 24-month-old rats were tested in the Morris water maze. Three weeks later animals were sacrificed and sections were processed for PSA-NCAM immunohistochemistry. Ageing was accompanied by an overall decrease in PSA-NCAM-immunoreactivity (IR) within the forebrain with a dramatic decrease of the number of PSA-NCAM-IR perikarya within the hippocampus and the piriform cortex. These results were confirmed by western blot analysis. No difference was observed in aged rats with or without spatial learning impairment. It is concluded that PSA-NCAM expression is dramatically reduced during aging, very likely reflecting a decrease in neurogenesis. Although these PSA-NCAM changes parallel the decrease in cognitive deficits, our data did not evidenced a causal relationship between these two parameters.

**797.10**


The changes of calcium homeostasis have been noticed to be one of an important aspect responsible for morphological and molecular deterioration in aging retina. As retina in young human has not yet been investigated from the point of calcium homeostasis. A high affinity calcium binding protein, calbindin has been identified from retina which is densely distributed in synaptic terminals besides retina and various subsets of cortical neurons was detected in the retina aging from 2 weeks to 30 months old rat. These changes were compared to that of another family member of calcium binding protein calbindinD28K, in the retina as well as in the visual cortex. The synaptic vesicle proteins, synaptophysin and synaptotagmin were also detected in the retina and cortex as marker proteins of synaptic function in aging neurons. Both synaptic marker proteins showed an age-dependent decrease only in the retina while calbindin densely distributed in the inner plexiform layer did not show any change in aging retina. However, calbindinD28K, localized at the outer plexiform layer showed a comparable change in synaptic marker proteins in the retina. A dramatic decrease in immunopositive bands in the inner plexiform layer which started after 12 month old was the most prominent change in aging retina, while in the cortices including occipital cortex no decrease in age-observered decrease was evident in synaptic marker proteins and calbindinD28K but not calbindin in the retina. On the contrary, in the cortex, none of these molecules showed age-dependent change. The role of these changes in aging retina remains to be disclosed.
797.11
INCREASING AGE ALTERS TRANSBLASTYL FLUIDITY AND CHOLESTEROL ASYMMETRY IN SYNAPTIC PLASMA MEMBRANES OF CA2+ DEPENDENT NURSE-SENSES IN THE HIPPOCAMPUS.

Previously, age differences on membrane structure have reported on the total or average change in membrane structure. The present experiments determined fluidity and cholesterol distribution of the exofacial leaflet of the synaptic plasma membranes (SPM) from 4-5, 14-15, and 24-25 mo old C57BL/6NNia mice using trioleinbenzenesulfonic acid quenching techniques and fluorescence microscopy. The exofacial leaflet of SPM from young mice was significantly more fluid as compared to the cytofacial leaflet. The large difference in fluidity between the two leaflets was abolished in SPM of the oldest age group. Age differences. The exofacial leaflet contained substantially less cholesterol than the cytofacial leaflet (15% vs 87%, respectively) in SPM of young mice. This asymmetric distribution of cholesterol was significantly modified with increasing age. There was approximately a two-fold increase in exofacial leaflet cholesterol in the oldest group when compared with the youngest age group. The bulk SPM cholesterol/phospholipid molar ratio did not differ among the three age groups. Transblastyl fluidity and cholesterol asymmetry were altered in SPM of older mice. This approach is a new and different way of viewing how aging modifies membrane structure. Age differences in SPM leaflet structure may contribute to the manifestation of membrane function. Supported in part by AG11056 and Dept. of Veterans Affairs.

797.12
DISTRIBUTION OF OMP mRNA IN THE CILIARY-DENDRITIC, SOMAL AND AXONAL COMPARTMENTS OF HUMAN OLFACTORY RECEPTOR NEURONS. T.J. Finch*, N.S. Rama Krishna, O.J. Buskova, E.L. Margarit, and M.J. Gotchell. Dept. of Physiology and ENT Surgery, Univ. of Kentucky College of Medicine, Lexington, KY 40536, and Roche Institute of Molecular Biology, Nutley, NJ 07110.

Differential localization of mRNAs in subcellular compartments may indicate a specific role for extracellular protein synthesis in neuronal function. The localization of OMP mRNA and protein was examined in olfactory receptor neurons. This mRNA was obtained as autopsy from humans who ranged in age from 26 weeks of gestation to 85 years of age, including three subjects with Alzheimer’s disease. Quantitative in situ hybridization was performed on 10 µm-thick sections using 32P-labeled antisense cRNA probes as described previously (Buskova et al., Genomics 20:452, 1994; Rama Krishna et al., Neuroreport 6:817, 1995). The mean grain density was 1.7 X (± 0.2) in the ciliary-dendritic, 4.0 X greater in the somal and 2.6 X greater in the axonal compartments than in the background. Also, the olfactory nerve layer-glomerular compartment in the olfactory bulb had a substantially higher grain density than these background. The mean grain density in the somal compartment increased systematically with age except in subjects with Alzheimer’s disease whose significance was less than in age-matched controls. The localization of grains over the compartments corresponded to the presence of mature olfactory receptor neurons as determined by OMP immunoreactivity at all ages studied. Our results indicate that OMP mRNA is located in the ciliary-dendritic, somal and axonal compartments of human olfactory receptor neurons and that OMP mRNA is substantially reduced in subjects with Alzheimer’s disease.

Supported by NIH grants DC 0019 (TVG) and DC 01715 (MLG).

797.13

The expression of heat shock proteins by the brain helps it to cope with rapid changes in its environment such as exposure elevated temperatures, oxidants and toxins. In this study, we have assessed the expression of two heat shock proteins (hsp), a constitutive form (hsp70) and an inducible form (hsp72), in the brains of adult (3 month old) and aged (18, 24 month old) rats. The levels of hsp70, quantitated by Western blotting, in the 18 and 24 month old rats were 92.2 ± 8% and 83 ± 20% (mean ± SE) of the 3 month old, respectively. The basal levels of hsp72 for the 18 and 24 month old animals were 103 ± 3 and 124 ± 8% of 3 month old, respectively. Exposure of these different groups of rats to heat stress (37°C, 1 h) resulted in substantial age-related differences in the induction of hsp72 following 8 h of recovery. Increses in hsp72 elicited by heat stress were 815 ± 17%, 202 ± 14% and 101 ± 8% compared to the naive 3, 18 and 24 month old control, respectively. Increases in hsp70 were negligible under identical conditions, being 109 ± 6%, 100 ± 5% and 107 ± 6% of the respective naive age-matched controls. These studies demonstrate that the aging brain has a markedly reduced response to stress, which might make it more susceptible to damage following adverse changes in its local environment.

797.14
THE DISTRIBUTION OF UBQUITIN-PROTEIN CONJUGATES IN VARIOUS REGIONS OF RAT BRAINS. E. R. Migos, Department of Biology, Michigan State University, Moorhead, Minnesota 56563.

The accumulation of varied molecular compounds is seen during the course of normal aging. In addition, specific lesions are associated with various age-related neuropathologies. These age-associated accumulations may, in part, be the result of alterations in proteolytic processing. The ATP-dependent ubiquitin proteolytic system is one candidate for such changes. Studies were performed using young (1 to 3 months old) Sprague-Dawley rats, both male and female. Brain tissue was isolated from cortex, cerebellum, hippocampus, striatum, and thalamus. Analysis of proteins was done using SDS-PAGE and immunological identification with anti-ubiquitin antibodies. Profiles from the different brain regions were determined in order to establish baselines for future comparison to aged brain samples.

797.15

Iron accumulation and loss in the aging brain may be caused by chronic exposure to free radicals. The generation of free radicals is rate-limited by the availability of iron stored within cells by ferritin. We have compared the distribution and intensity of immunostaining for ferritin in the hippocampus of two-year-old male rats (n=7) male rats to rats which were euthanized with sodium pentobarbital (60mg/kg IP), perfused transcardially with 4% paraformaldehyde in phosphate buffer (pH 7.2), and their hippocampal formations sectioned at 100µm on a vibratome. Sections were incubated in L-chain ferritin antisera for 5 days (1:2500; IOD), then in secondary antisera and finally with streptavidin-HRP-DAB. In adolescent rats, cells with weak ferritin immunoreactivity are common in the stratum radiatum of CA2, but not elsewhere. The most significant differences between individual rats in the total numbers of labeled cells (p<0.1; 2-tailed t-test), in old rats, the hippocampus contains more L-chain ferritin than any other brain region. Large numbers of strongly labeled cells are found in the stratum radiatum of CA2, CA3, and CA4, and to a lesser extent, the subiculum. The total number of ferritin-immunoreactive cells in old hippocampus is 6.5-fold higher than in adolescent hippocampus (p<0.001; 2-tailed t-test), with CA4 showing a 78-fold increase in the number of cells containing ferritin immunoreactivity. Subicular number of old rats (r=0.05; 2-tailed t-test). Comparisons with different titrations of the primary antisera indicate that the maximal extent of ferritin accumulation is an area of magnitude higher than in adolescent hippocampus. At both ages, most ferritin-immunoreactive cells resemble reactive microglia. Microglia may be implicated in age-related hippocampal degeneration through their storage of iron and aluminum, or through an associated production of free radicals.

Supported by grants to SRR from the ARC and Mayne Bequest Fund.
977.17
EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN SPINAL MOTOR NEURONS AFTER AXOTOMY AND IN AGING F344 RATS. O. Hanson-Painton*, P. Grammas and J.M. Jacob. Deps. of Pathology and Anat. Sci., Univ. of Oklahoma HSC, OKC, 73190.

Nitric oxide (NO) has been shown to be both an important neuromodulator and neurotransmitter in the CNS. The objective of this study was to examine the effects of aging and axotomy on iNOS expression in spinal motor neurons. In 6 month F344 rats, the sciatic nerve was cut and iNOS expression examined at 1-7 days after axotomy. The retrograde tracer Fluorogold was used to locate the motor columns supplying the right sciatic nerve. Using a polyclonal antibody directed against iNOS and avidin-biotin enhanced DAB to visualize protein immunoreactivity (IR), the distribution and temporal expression of iNOS-IR was determined. The data demonstrate iNOS-IR was increased by day 3, by day 7, iNOS-IR was reduced but still above control levels. Also, a comparison of 6 and 24 month spinal motor neurons showed a marked increase in iNOS-IR. These data demonstrate an upregulation of iNOS after axotomy and in aging, suggesting increased production of NO with axonal injury and age. Supported by grants to PG (NS 30457) and JMJ (AFAR).

978.1
A CALCIUM-DEPENDENT CHLORIDE CURRENT IN MOUSE SYMPATHETIC NEURONS. F. de Castro, E. Góez-Barrionuevo and R. Gallagé*. Instituto de Neurociencias y Departamento de Fisiología, Universidad de Alicante, 03080-Alicante, Spain.

Anatomized rat sympathetic neurons show a depolarization after spike firing (ADP), which is generated by a calcium-dependent chloride current (Sánchez-Vives & Gallagé, J. Physiol. 475: 391, 1994). In normal cells this conductance may be located in the dendrites and only become evident in somatic recordings after the dendrites are cut by axotomy. Therefore, we have investigated whether this current is present in non-anatomized mouse sympathetic neurons which have shorter dendrites than similar cells in the rat. The experiments were done in an “in vitro” preparation of the superior cervical ganglion using single-electrode current- and voltage-clamp techniques and intracellular staining with neurobiotin. In the presence of TTX (1 μM) and TEA (25 mM), inward and outward currents were recorded during 50-200 ms depolarizing pulses from -50 mV, followed by slowly decreasing inward tail currents that lasted 400-800 ms. The tail current was blocked by anisomycin-9-carboxylic acid (2 mM), a selective blocker of chloride channels, and its reversal potential shifted in accordance with the Nernst equation when the extracellular chloride concentration was changed. Calcium-free solution or CsCl (200 mM) abolished both, the inward peak and the tail current. In current-clamp recordings, a train of spikes evoked a marked ADP in the majority of the cells. The amplitude of the ADP for a given neuron was inversely correlated with the number and total length of dendritic branches. These results suggest that mouse sympathetic neurons have a calcium-dependent chloride current localized in the distal dendrites. Supported by Grant PB92-0347 from the DGICYT (Spain).

978.2
SEROTONIN-ACTIVATED CI CURRENT IN RAT BRAIN STEM NEURONS. R.A. Davidoff*, J.C. Hackmann*, S.R. Whitmore and A.Y. Valeyu*. Neuropsychology Laboratory, Veteran’s Administration Medical Center, Department of Neurology and Miami Project I University of Miami, Miami, Florida 33101, USA.

We studied RN46A cells, a serotonergic neuronal cell line derived from E13 rat embryos (White et al., J. Neurosci. 1994). Intracellular recordings in the whole-cell configuration were made at room temperature using CsCl-filled micropipettes. 5-HT receptor agonists and antagonists were applied either by pressure pulses from closely positioned pipettes or by gradual diffusion in the extracellular medium. Application of 5-HT evoked a dose-dependent inward current when holding membrane potentials were negative. When the equilibrium potential for CI was set near 0 mV, current responses to 5-HT reversed polarity near 0 mV suggesting a dominant role for CI ions in the conductance response. Bicuculline and strychnine had no effect on 5-HT activating current while picrotoxin blocked the CI current. The slow kinetics of activation of the CI conductance suggest the participation of two messengers in this process. (Supported VAMC MRIS #1769 and 3369 and USPS ANS 17577).

978.4

King and Carpenter (Neurosci. Lett. 22:343-348, 1987) observed that transmitter-induced CI dependent responses in Aplysia neurons often “interact” with one another: they are cross-desensitizing, and are often blocked by the same antagonists. We have studied the CI-dependent responses of the medial cells of the pleural ganglion to fast perfusion application of ACh, GABA and Glutamate. None of these responses, studied in whole-cell patch clamp, could be selectively blocked by the antagonist tested (strychnine, picrotoxin, bicuculline, and tubucurarine). Furthermore, each of these transmitter-induced responses desensitized with prolonged agonist application, and a “desensitizing” pulse of any one of the three transmitters caused a significant cross-desensitization of the responses induced by the other two. Even when using ATP- GTP-free solutions in very low resistance whole-cell pipettes, all three responses, as well as their cross-desensitizing interactions, persist. In spite of the marked interaction between the GABA response and those elicited by ACh and Glutamate, GABA response appears to be mediated by a different channel. When sulphate was used as the anion in the pipette solution, the GABA response was reduced by about 70-80% whereas the responses to ACh and Glutamate remained unchanged. In contrast, when CI was used, all three responses, measured at a fixed number of mV less negative than ECI, remained unchanged. It thus appears that the interactions between these transmitter-induced responses are not simply the result of a common activation of a single receptor-channel complex.
798.5

SELECTIVE ANION PERMEABILITIES DISTINGUISH SUBCELLULAR CALCIUM POOLS IN RAT BRAIN.

Stephen L. Fascella and Aiy. Yenmei* Dept. of Neurology and Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

The anion selectivity of rat brain intracellular, nonmitochondrial 4Ca+ sequestering compartments was studied using rat brain microsomal fractions and fresh frozen sections. Mg-ATP-dependent 4Ca+ transport in sodium acetate containing buffers showed a strict requirement for anion cotransport as no 4Ca+ uptake occurred in buffers containing the impermeant gluconate as the sole anion. Prominent 4Ca+ accumulation was observed with oxalate, fluoride and phosphate as the sole anions and intermediate accumulation using acetate or chloride. By using a number of Ca+ transport inhibitors two distinct compartments could be distinguished, each showing unique anion preferences. Thapsigargin (Tg), cyclopiazonic acid (CPA) and 2,5-di(tert-butyl)hydroquinone (IBQ) potently blocked oxalate, fluoride, or phosphate supported Ca+ transport. Inhibition of Mg-ATP dependent chloride supported 4Ca+ uptake, however, required much higher concentrations of these inhibitors. Autoradiographic localization of 4Ca+ sequestering compartments using fresh frozen rat brain sections revealed unique anatomical distributions for the chloride selective pool with enrichment in brain stem, deep cerebellar nuclei and spinal cord. 4Ca+ uptake supported by oxalate, fluoride or phosphate was instead much more prominent in forebrain structures and cerebellar cortex.

798.7

SWELLING-INDUCED AMINO ACID EFFUX IN THE HUMAN NEUROBLASTOMA CELL LINE CELL CHP-100. S. Basuapati, C. Huang, A.W. Mangal*, K. Kirk, P.N. Leech* and J.C. Ellory.* University Laboratory of Physiology, University of Oxford, Oxford OX1 3PT, UK. Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, NC, USA, and Department of Neurology, Institute of Psychiatry, London SE5 8AF, UK.

Pathological disturbances (such as hypotension or ischemia) may result in significant changes in neuronal cell volume which have not been investigated in detail. The present studies evaluated the effects of hypotensive stress on cell volume and amino acid efflux in the human neuroblastoma cell line CHP-100. Using a Couche Multiuser, CHP-100 cells were found to swell by ~35% ± 5% when the osmolality of the extracellular solution was decreased from 290 to 190 mOsm/kg H2O. The rapid swelling was followed by regulatory volume decrease (RVD) with the cell volume approaching the isosmotic value over 15 min. The cells loaded with the calcium-sensitive dye Fura-2, a similar hypotensive shock caused a slow taurine efflux to 299 ± 22% (p<0.05) of control values. This efflux was inhibited by the chloride blockers 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), 4,4'-diisothiocyanato stilbene-2,2'-disulfonic acid (DIDS), and reftile acid, and was also dependent upon extracellular calcium. Hypotensive stress also caused a significant increase in Vγ-glycine efflux, that was sensitive to NPPB. By contrast, efflux of H-glutamate was not significantly affected by hypotensive exposure. It is concluded that CHP-100 cells undergo RVD when confronted with hypotensive stress and of taurine and glycine may contribute to this via a calcium-dependent anion permeability pathway.

798.9

MECHANISM OF ACTION OF FLUFENAMIC ACID ON Ca-ACtIVATED CHANNELS. I. D. Partridge, R. J. Lewis, T. Shaw. Dept. of Physiol. & Path., Univ. of New Mexico, Albuquerque, NM 87131

Non-steroidal anti-inflammatory drugs (NSAIDs) are well known to produce anti-inflammatory effects through actions on prostaglandins, but their mechanism of analgesic action is less well understood. We have shown previously that the NSAID, flufenamic acid, transiently activates both a Ca-activated nonselective (Ica) and a Ca-activated outward (Iow) current in neurons and that this is paralleled by a maintained rise in [Ca2+]. These effects are specific and not shared by two other fenamates. In this study, we investigated the mechanism of action of fena on [Ca2+] and these two currents. A rise in [Ca2+] is still observed following fena application in dual-chamber or thapsigargin but the transient increase in Ica and Iow is reduced. Application of protein kinase inhibitors and membrane permeable CAMP analogs suggest that the effect on membrane currents is through channel phosphorylation. Both the [Ca2+] response and the current activation process were fully recovered after a 20 min wash following fena application. We conclude that fena causes a release of Ca from mitochondria and ER that activates these Ca-activated currents and that these stores can be rapidly refilled. The reduction in Iow in the presence of a maintained elevation of [Ca2+] does not appear to result from channel phosphorylation. These are the first descriptions of the mechanism of action of this NSAID on neuronal Ca-activated channels. These actions may underlie some of the analgesic actions of this drug.

798.10

CALPAIN ACTIVATED CHLORIDE CHANNELS IN EXCISED PATCHES OF MYOTUBES AND NEURONS: G. C. McCarter and R. A. Steinhardt. Dept. of Molecular and Cell Biology, Univ. of Calif., Berkeley, CA 94720.

We have previously reported that exogenous calpain activates chloride channels when applied to the cytoplastic face of about half of excised rat myocyte membrane patches (FKA) transformed with a similar activation in cerellar granule cells and provide evidence that a membrane-resident protease may be responsible for activating the channel when it arises spontaneously. The channel in both cell types is outwardly rectifying, has a conductance between 30 and 90 pS and is blocked by 5-nitro-2-(3-phenylpropylamino)benzamide (NPPB), similar to channels seen in cells from airway epithelium and other tissues. Channels are also activated when bath calcium is raised to 0.18 to 1.8 mM, again in about half of excised myocyte patches, and this effect is blocked by the presence of the protease inhibitor leupeptin. Cytochalasin D has been reported to activate chloride channels in myoballs and other cells and we report here such an activation in neurons. This and the fact that cytoskeletal proteins are known to be calcium substrates suggest that proteolytic activation of the chloride channel may be mediated via a cytoskeletal regulation of the channel.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
T70.1
DEVELOPMENTAL EXPRESSION OF AN INWARDLY RECTIFYING CHLORIDE CONDUCTANCE IN RAT BRAIN.

A voltage sensitive, inwardly rectifying chloride channel, which has been shown to play a significant role in the modulation of neuronal responses to GABA activation, is present in the same neurons that express mRNA for a previously cloned chloride channel (CIC-2) with similar properties. These results strongly suggest that CIC-2 is the source of the inwardly rectifying conductance. Using dihydrogen labeled riboprobes, we have examined the expression of CIC-2 during postnatal development of rat brain and have found rapid changes in the pattern of developmental expression of CIC-2. In hippocampus, in particular, expression is more widespread in neonatal animals than in adult rats and can be found in proliferative layers. Similarly, CIC-2 was expressed in neurons of caudoputamen in the early stages of post natal development but was essentially absent in adult animals. In contrast, in cerebellum, the expression of CIC-2 is a relatively late post-natal event occurring between 7 and 14 days after birth during the period of synapticogenesis. To summarize, CIC-2 is expressed extensively in developing rat brain neurons including cells within germinial layers and becomes more restricted in the adult. These results suggest that CIC-2 may subserve functions which are different during migration and synaptogenesis than in the adult.

CAULCIUM CHANNELS: MISCELLANEOUS BLOCKERS

T70.2

We have recently reported that neuronal Ca2+ channels were blocked by the peripheral-type benzodiazepine receptor (PBR) agonist Ro 5-4864, whereas they were activated by the nortropic nefiracetam (DM-9384), although this compound has a binding affinity to PBR. In the present study, we have further investigated the effects of nefiracetam on the Ca2+-channel and PBR by using various nortropic agents. Ca2+-channel currents were optimally enhanced by nefiracetam at 1 µM and less potently by aniracetam at 10 µM. The currents were not affected by oxiracetam or piracetam, or chemically-unrelated cognitive enhancers such as idebenone and bifemelene. Unlike nefiracetam and aniracetam, these compounds inhibited the currents at higher concentrations. Binding of HR-PK 11195, a PBR antagonist, to the mouse brain was inhibited by nefiracetam and aniracetam with IC50 of 0.39 and 1.59 nM, respectively, whereas it was not inhibited by oxiracetam and piracetam. The results suggest that PBR is a low-affinity site for nefiracetam and aniracetam and may be involved in their drug actions at high concentrations, whereas PBR is not involved in drug actions of other cognitive enhancers such as oxiracetam and piracetam.

T70.3

We have recently reported that the cognitive enhancer, nefiracetam (DM-9384), increases L-type Ca2+ channel currents in NG108-15 cells and that the effect is inhibited by pertussis toxin (PTX), suggesting that inhibitory G-proteins (Gi/Go) mediate the drug action (Yoshii and Watabe, Brain Res. 642, 123-131, 1994). In this study, we have further examined whether stimulatory G-proteins (Gs) are involved in the action of nefiracetam using the whole-cell patch-clamp technique. Under the condition that L-type currents were blocked by nifedipine (10 µM), the remaining high voltage-activated-long-lasting currents were recorded (50 nM), which is known to activate Gi/Go, reduced the currents in a PTX-sensitive manner. The inhibited currents were reversed by nefiracetam (1 µM). On the other hand, which is known to activate Gi/Go (10 µM), did not affect the currents. The inhibited currents by PGE1 were not recovered by nefiracetam. The results suggest that the nefiracetam action is mediated by inhibitory G-proteins (such as Gi/Go), but not by stimulatory G-proteins (Gs).

T70.4

Lifarizine (RS 87476) is a use- and voltage-dependent sodium channel blocker (Br. J. Pharmacol., 113:600, 1994) which also blocks the effects of calcium channel activators in smooth muscle (Br. J. Pharmacol., 104:1506, 1991) and is neuroprotective (Radiology, 179:221, 1991). We examined the effects of lifarizine on several classes of neuronal calcium channel in both rat and human tissue. In rat SCG cells, using a holding potential of -100 mV, 10 µM lifarizine blocked 70±2% (n=3) of the L-type resistant current while 1 µM blocked 25±1% (n=6). The N-channel blocker conotoxin GVIA blocks approximately 70% below the same conditions. When the holding potential was lowered to -50 mV, the block by 1 µM lifarizine significantly increased to 74±6% (n=3). Against P-type calcium channels in acutely dissociated rat cerebellar Purkinje cells 10 µM lifarizine blocked 53±7% (n=3) of the o-Aga-IVA-sensitive current at a holding potential of -80 mV. The effects of lifarizine on human calcium channels were tested using fura-2 fluorescence measurements in the human IMR32 neuroblastoma cell line. Lifarizine blocked the n-conotoxin GVIA-sensitive (N-channel) Ca2+-induced calcium increase with an IC50=3.4 µM and nifedipine-sensitive (L-channel) increase with an IC50=0.8 µM. At 10 µM, lifarizine also blocked 98% of the current remaining in these cells after treatment with saturating concentrations of both nifedipine and GVIA. These data indicate lifarizine is a non-specific, voltage dependent blocker of neuronal calcium channels. Lifarizine, along with its ability to block voltage sensitive sodium channels, may play an important role in its neuroprotective activity.

There are a variety of subtypes of voltage-gated Ca²⁺ channels that have been described using electrophysiological and biochemical techniques. One subtype of Ca²⁺ channels, designated as N-type, is found predominantly on neurons and has been shown to be important in mediating neurotransmitter release as well as neuronal excitability. The Ca²⁺ channel expressed in Xenopus oocytes (mainly B and some HVA Class B) have pharmacological and biochemical properties that are similar to N-type Ca²⁺ channels. Voltage-dependent blockers of N-type Ca²⁺ channels may be useful in the treatment of neurological disorders, including stroke.

Microinjection of cRNA encoding for human neuronal α₉, human neuronal β9 and rabbit skeletal muscle α₂ subunits produced high-voltage-activated Ca²⁺ channels in Xenopus oocytes, whose activity was measured using two-electrode voltage-clamp techniques. The block of expressed channels by lidocaine and verapamil was done at holding potentials where there was a small degree (5%) of steady state inactivation (480 nV) and at depolarized holding potentials (-55 mV) where approximately 50% of the channels were inactivated. Using these two holding potentials we found a 5-10 fold increase in the potency of these two compounds when inducing current from the more depolarized holding potential. The α-conotoxin GVIA tested in the same paradigm blocked Class B Ca²⁺ channels, but showed no voltage-dependence. Lidocaine and verapamil are non-selective ion channel blockers, producing voltage-dependent blocks of sodium and L-type calcium (verapamil) channels.

These results suggest that lidocaine and verapamil interact with a common site on Na⁺ and Ca²⁺ channels that is revealed under depolarization or inactivation.


The extract of Cissus sicyoides (Ca) from dry leaves 1:10 w/v produces vasoconstriction in aortic rings without endothelium in a dose dependent manner (1). As this could be explained by calcium mobilization through voltage dependent channels or receptor dependent channels, we tested several potassium chloride (KCI) concentrations. KCl 7.7-10 molar increased Ca contractility while KCl 20-77 molar decreased this response. Ca vasorelaxation dose response curve may be observed in calcium free solutions with EGTA 0.2 mM and 3 mM and caffeine 5 and 50 mM. Caffeine blocked Ca contraction dose dependent. These suggests that Ca contraction depends on calcium channels activated by membrane voltage changes and on internal calcium deposits.


PROPOFOL INDUCES EXTRACELLULAR ACIDIFICATION IN NEURONS. K. Delmestroh, H. Eriksson, Â. Schiönder, P. Sjölander, P. Eentell. Departments of Anaesthesiology and Cell Biology, Uppsala University Hospital, S-581 85 Linköping and Astra, Södertalje, Sweden.

The site of action of the anesthetic drug propofol (Diprivan®) is still unknown. We have previously shown that propofol induces changes in the cytoskeleton arrangement after an intracalicular calcium rise (1). Our hypothesis is that anesthetic drugs interact with the cell membrane which change the state of the ion channels and induce changes in the cytoskeleton.

We have used a microphysiometer (2) to measure the amount of proton excretion in primary culture of neurons after stimulation with propofol in the concentration of 0.3, 3 and 30 μg/ml respectively. We used medium or 10% Intralipid® as controls.

To further investigate how the propofol-induced rise in intracellular calcium occurs we used tyrosine kinase inhibitor herbimycin A, incubated for 30 min, and thereafter measured intracellular calcium in single cells as described previously (1).

The results showed dose dependent and reversible changes in the extracellular acidification. We believe this acidification is caused by acid metabolites leaving the cell through open ion channels, probably calcium channels. The intracellular calcium rise was reduced when herbimycin A was added. The reduction was caused by reduced influx of calcium ions. This shows that tyrosine kinase has a role in the intracellular pathways caused by propofol.

GENETIC DISRUPTION OF THE M1 MUSCARINIC RECEPTOR IN MICE. S. E. Hamilton*, M. Qi, G. S. McKnight, N. M. Nathanson, R. L. Jeck. Department of Pharmacology, University of Washington, Box 357280, Seattle WA 98195-7280

The m1 muscarinic receptor is found in high concentrations in the hippocampus and is linked to the regulation of synaptic transmission. We have generated transgenic mice containing a deletion of the m1 receptor. This will allow us to understand the role of the receptor in synaptic function.

800.3

m3 MUSCARINIC RECEPTORS ON MICROGLIA: IMPLICATIONS FOR CHOLINERGIC-NEURON-MICROGLIAL COMMUNICATION. G. Ferrer-DiLeo and D.D. Flynn. Dept of Pharmacology, Univ of Miami School of Medicine, Miami, FL 33101

Microglia, the resident macrophages of the CNS, are involved in both protective and destructive processes important for neuronal survival. The close association of microglia with basal forebrain cholinergic neurons and the presence of cholinergic muscarinic receptors on microglia suggest possible unique cholinergic-neuron-microglial interactions. We have used an in vitro co-culture system to study the effects of muscarinic receptor activation on microglial function and the potential for microglial-neuron signaling. In this study, we have investigated the effects of m3 muscarinic receptor activation on microglial cell morphology, migration, and cytokine expression. Our results suggest that m3 receptor activation can modulate microglial function, providing new insights into the role of cholinergic signaling in the CNS.

800.4

SODIUM NITROPRUSSIDE INDUCES INTERNALIZATION OF MUSCARINIC RECEPTORS. B. Maggs*, P. Batters, F. F. Frentz, F. Vaghn and G. U. Gleim. Institute of Pharmacology, School of Medicine, University of Pisa, Italy.

In the present study, we have investigated the internalization of muscarinic acetylcholine receptors induced by sodium nitroprusside (SNP), a potent vasodilator. We have examined the internalization of m3 muscarinic receptors using a cellular radio-receptor assay and immunocytochemistry. Our results indicate that SNP-induced internalization of m3 receptors is concentration-dependent and time-dependent. These findings suggest that SNP may play a role in the regulation of muscarinic receptor function in the cardiovascular system.

800.5

CONSTITUTIVELY ACTIVE MUSCARINIC RECEPTORS PRODUCED BY RANDOM MUTAGENESIS OF THE SIXTH TRANSMEMBRANE DOMAIN. T. A. Staudenraus, E. S. Burstein, D. Hill-Eubanks, M. R. Brand. Department of Psychiatry, University of Vermont, Burlington VT 05405 and Receptor Technologies, Winooski, VT 05404

The m3 muscarinic receptor is a G protein-coupled receptor that plays a critical role in the regulation of cholinergic neurotransmission. We have used random mutagenesis to generate constitutively active m3 receptors that can activate downstream effector pathways in the absence of ligand. Our results indicate that these mutants can be used to study the signaling mechanisms of the m3 receptor and the role of m3 in the regulation of cholinergic neurotransmission.

800.6

NOVEL HIGH THROUGHPUT ASSAYS OF CLONED RECEPTOR PHARMACOLOGY IN LIVING MAMMALIAN CELLS. Terri L. Murger, Christine A. Staph, D. Harn, Brainerd, Ross Durbank, S. Penelope Jones*, and Mark R. Brann.

Receptor Technologies Inc. 275 East Allen Street Winooski, VT 05404

In this study, we have developed a high-throughput assay for the pharmacology of cloned receptors in living mammalian cells. Our assay offers several advantages over traditional methods, including the ability to perform experiments in a high-throughput format and the ability to study the effects of multiple ligands on the same cell line. Our results suggest that this assay can be used to study the pharmacology of cloned receptors in living cells, providing new insights into the role of these receptors in disease processes.

These studies highlight the importance of understanding the role of cholinergic signaling in the CNS and the potential for targeting m3 muscarinic receptors for therapeutic interventions. The development of high-throughput assays for the pharmacology of cloned receptors in living cells offers new opportunities for the study of receptor function.
Isolation and Characterization of the chick m2 and mouse m1 muscarinic acetylcholine receptor promoters.

Marc L. Rosenberg, Jai Wei, Robert A. Shager, Neil M. Nathanson

We have isolated genomic regions containing the putative chick m2 (cm2) promoter and the putative mouse m1 (m1) promoter.

Construction of recombinant promoters drive the expression of the firefly luciferase gene when transiently transfected into IMR-32 human neuroblastoma cells. Treatment of cells for 3 h, with leukemia inhibitory factor (LIF) or ciliary neurotrophic factor (CNTF) results in an increase in cm2 driven luciferase expression while m1 driven luciferase expression remains unchanged. Treatment with nerve growth factor (NGF) had no effect on expression of either reporter constructs. We have begun to compare the regulation of the endogenous mAChR levels and their respective mRNAs with the regulation of the reporter gene constructs.

Initial studies indicate the putative cm2 promoter will drive luciferase expression in transiently transfected chick heart primary cultures (>8 fold above vector alone). We are currently investigating the regulation of the cm2 luciferase constructs in this system.

Identification of Single Amino Acid Residues Determining Selective Activation of Gq by the m3 Muscarinic Acetylcholine Receptor.

Nel Binn, Jin Yan and J. Wess

A large body of evidence suggests that the specificity of receptor G protein interactions is determined by multiple intracellular receptor domains. To gain insight into the molecular mechanism governing receptor G protein coupling selectivity, specific amino acids which are of particular importance for proper G protein recognition need to be identified. Towards this goal, the present study was undertaken to identify single amino acids required for selectively coupling the m3 muscarinic acetylcholine receptor to G proteins of the Gq11 family. Distinct intracellular segments/peptides (SPs) which were systematically substituted into the structurally closely related m2 muscarinic receptor which does not couple to Gq11 proteins. The resulting receptors were expressed in COS-7 cells and studied for their ability to induce agonist-dependent stimulation of phosphatidyl inositol hydrolysis, a response known to be mediated by the molecular subunit of the Gq11 class. Using this approach, we identified four amino acids in the second intracellular loop and four amino acids at the C-terminus of the third intracellular loop of the m3 muscarinic receptor which are essential for efficient Gq11 activation. We could demonstrate that these amino acids, together with a short segment at the N-terminus of the third intracellular loop, fully account for the G protein coupling preference of the m3 muscarinic receptor. Taken together, our data strongly suggest that only a rather limited number of amino acids, located on different intracellular regions, are required to define the functional profile of a given G protein-coupled receptor.

Site-Directed Mutagenesis of the m1 Muscarinic Receptor: Role of B-S-X-B Motif in Receptor-G Protein Coupling.

N.S.M. Geoghegan, R.T. Glise, C.M. Fraser and N.J. Leg

Seven transmembrane domain (7TMD) receptors mediate the activity of their effector enzymes via G-proteins. Examples of single tmd receptors that functionally interact with G-proteins are all but known.

Of interest is the finding that the cytoplasmic regions of these two structurally diverse class of receptors contain a B-S-X-B motif (where B is a basic amino acid and X is any other acid) which is postulated to be the G-protein activating domain. Agonist-stimulation of m1 muscarinic acetylcholine receptors (m1mAChRs), belonging to the 7TMD receptor family, results in hydrolysis of phosphatidylinositol (PI) and activation of adenylyl cyclase.

The KAAA [365] motif (where the number indicates the position of the last amino acid) in the 2nd and 3rd intracellular loops, respectively. We have mutated the basic amino acids to alanine (A) to access their role in receptor-G protein coupling. Antagonist binding and expression levels (1-2 pmol/mg) of the resulting mutants were all comparable to wild-type mAChRs.

The AAAAAA [365] mutant did not exhibit any agonist-stimulated PI hydrolysis. The KAAA [365] mutants exhibited decreased affinity and efficacy to stimulate PI hydrolysis compared to wild-type mAChRs, respectively. The KAAAA [365] and KAAA [365] mutants exhibited both decreased G protein coupling and PI hydrolysis. These data demonstrate that the KAAA [365] motif is the G-protein activating domain with each basic amino acid contributing to agonist-stimulated PI hydrolysis.

Characterization of a Chick m3 Muscarinic Acetylcholine Receptor. S. Crognier, K. Tietze and N.M. Nathanson

A partial length cDNA clone coding for a muscarinic acetylcholine receptor was isolated from chick heart using PCR and degenerate oligonucleotide primers. Sequence analysis showed this fragment to have 91% similarity and 88% identity with the third cytoplasmic loop of the rat m3 receptor. This fragment was used to probe a chicken genomic library to obtain a full length clone containing the (cm3) muscarinic receptor. The cm3 receptor, like all other vertebrate muscarinic receptors cloned to date, did not contain any introns in the coding region of the gene. cm5 specific oligonucleotides were used in reverse transcription PCR assays to demonstrate that the cm5 receptor is expressed in both chick heart and chick brain.

When expressed in stably transfected CHO cells, the cm5 receptor exhibited high affinity for the muscarinic receptor specific antagonist 3H-NBQ. Lindag binding assays with perinazpine, AFDX-116, and carbamol show this receptor to have pharmacological properties similar to other cloned muscarinic receptors. Functional studies demonstrate the ability of this receptor to stimulate phospholipase C and increase carbachol stimulated phosphatidylinositol hydrolysis in stably transfected CHO cells.

Structure of the m4 Muscarinic Receptor Gene and its Promoter. Arvee Roopra, Ian C. Wood, Christina A. Harrington and Noel J. Buckley

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G-protein coupled receptors are encoded by one of the most diverse gene families in the mammalian genome, accounting for as much as 1% of the entire genome. Most, if not all, of these genes have a unique contribution within the nervous system and hence is of interest to determine how these expression profiles are brought about i.e. What determines the receptor repertoire of individual neurons?

Cholinergic muscarinic receptor genes are members of this gene superfamily. The m4 gene is known to be expressed in the CNS, autonomic ganglia and lung. Activation of m4 receptors can lead to closing of N-187; a calcium voltage sensitive Ca2; channel and activation of adenyl cyclase. Analysis of the m4 gene has shown that it contains a 400bp non-coding exon separated from the single coding exon by a 4.8kb intron. A rat cDNA clone containing the m4 gene and 20kbs of upstream sequence was found to be sufficient to direct expression of the mouse gene in a cell type specific manner when transfected into cell lines. A DNA sequence analysis of the 5'-flanking region of the m4 promoter showed it to contain the putative regulatory sites.

Cellular mechanism and receptor type responsible for the muscarinic-induced currents in dorsolateral septal nucleus (DSLN) neurons were investigated by using slice patch-clamp technique. Bath-application of muscimol (3-100 μM) caused either inward current (I_m) or outward current (I_om) responses. These currents were associated in increase in membrane conductance and were voltage-independent. The reversal potentials of I_m and I_om were ~170 to 5.3 mV (n = 14) and -90.4 ± 3.7 mV (n = 9), respectively. In subpopulations of DSLN neurons, muscarinic caused the inward current by suppression of a voltage-dependent, non-inactivating K⁺ current, the M-current. The I_m was concentration-dependent; the I_m was completely reduced both I_m and I_om. Pirenzepine (2.0 μM) also reduced the I_m and the I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both I_m and I_om. Pirenzepine (2.0 μM) also reduced both I_m and I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both I_m and I_om. Pirenzepine (2.0 μM) also reduced both I_m and I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both I_m and I_om. Pirenzepine (2.0 μM) also reduced both I_m and I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both I_m and I_om. Pirenzepine (2.0 μM) also reduced both I_m and I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both I_m and I_om. Pirenzepine (2.0 μM) also reduced both I_m and I_om in a competitive manner. Similar results were obtained in a similar manner with KCI 60 mM (n = 10). M4 (2.0 μM) completely depresses both


800.1 MUSCARIC RECEPTOR INHIBITION OF AGONIST-STIMULATED CYCLIC AMP IN GUINEA PIG ILEM. L. J. Grace and F. J. Elbert. Dep. of Pharmacology, University of California, San Francisco, CA 94143.

The longitudinal muscular of the guinea pig ileum contains both M subtypes of muscarinic receptors. The M2 subtypes couple to phosphoinositide hydrolysis and elicit a direct contraction, whereas the more abundant M1 subtype inhibits adenylate cyclase and has an indirect role in contraction. In rat ileum only forskolin, isoproterenol, and PGE2 increased cAMP accumulation, and of these, only responses elicited by forskolin and isoproterenol could be opposed by the M2 receptor (Griffith et al., J. Pharmacol. Exp. Ther., 263(1): 221-9, 1992). The purpose of this study was to define the relative contributions of the guinea pig ileum. We investigated the ability of oxotremorine-M (oto-M) to inhibit cAMP accumulation in slices of the guinea pig ileum in the presence of various agonists known to stimulate adenylate cyclase. The accumulation of cAMP (50% over basal) was observed with maximal concentrations (10-10 M) of forskolin, isoproterenol, PGE2, PGI2, and PGI4, with PGE2 being the most effective in basal conditions. Moderate cAMP accumulation (50-50% over basal) was seen using dopaminergic agents, 5-HT, 5-methoxytryptamine, dimaprit and VIP. Little or no effect (<25% over basal) was observed with SKF-38397, 2-chloroadenosine, cibenzoline, P22, secretin and vasopressin. Oto-M (1uM) inhibited cAMP accumulation by 35% under basal conditions, while forskolin- and isoproterenol-stimulated cAMP was inhibited by 73 and 61%, respectively. Oto-M inhibited PGE2- and PGI2-stimulated cAMP by 48 and 56%, respectively, but only inhibited PGI2-stimulated cAMP by 31%, a value no greater than the oto-M effect on basal cAMP. Oto-M inhibition of cAMP accumulation in the presence of the other agonists with moderate or no stimulation of adenylate cyclase was less than or equal to magnitude to its effects on basal cAMP. Our results show that only forskolin, isoproterenol, PGE2, and PGI2, cause an appreciable increase in cAMP that is opposed by muscarinic stimulation, suggesting that the relaxing effects of these agents may be specifically inhibited by the M2 receptor. (Supported by NIH grant NS09002)

800.2 Sequestration of muscarinic acetylcholine receptors. H. Tsuga, E. Okuno, K. Kameyama, and T. Haga, Inst. for Brain Research, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Sequestration of muscarinic acetylcholine receptors (m1-m5), which was assessed as loss of [3H]methylyscopolamine binding activity from the cell surface, was examined in COS-7 cells that had been transiently transfected with M1, M2, M3, M4 or M5. Co-expression of G-protein coupled receptor kinase (GRK2 = FARK1) or GRK2 dominant-negative mutant (DN-GRK2). The agonist-dependent phosphorylation and sequestration of m2 receptors were facilitated by co-expression of GRK2, and reduced by co-expression of DN-GRK2. The sequestration of m4 receptors was observed by treatment with 10-5 M or higher concentrations of carbacholamine and 40% of m4 receptors were sequestered by treatment with 10-3 M of carbacholamine for 2 hours. Co-expression of DN-GRK2 reduced the sequestration of m4 receptors to less than 25%. For m1, 3, 5 receptors, only less than 25% of receptors were sequestered by treatment of 10-5-10-3 M of carbacholamine for 2 hours. These results indicate that the phosphorylation of m2 and m4 receptors by GRK2 facilitates their sequestration and the mechanism of sequestration may be different between m2, 4 receptors and m1, 3, 5 receptors.

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801.7 NEOSTIGMINENAPPEARS TO ACTIVATE MUSCARINIC RECEPTORS ON MUPPUPPARASYMPATHETIC POSTGANGLIONIC NEURONS.
J.H. Cardenas, Backman*.
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Indirect evidence from in-vivo studies in cats suggests that the bradycardia produced by nicotine is mediated by activation of the acetylcholine scopolamine may be mediated, in part, by direct activation of muscarinic receptors on cardiac parasympathetic postganglionic neurons (J Pharm Exp Ther 1993:269:74). In the present investigation, this possibility was examined more directly by studying the effect of direct application of neostigmine to individual parasympathetic postganglionic neurons of the mupuppy (Necturus maculatus) cardiac ganglia. Previous studies in this laboratory have shown that muscarinic agonists activate an inwardly rectifying potassium conductance via m2 receptors (Neuropharmacology 1992:31:331). Neostigmine and uncurare-induced hyperpolarizations were reversibly inhibited by both non-selective (atropine 1 pM) and selective m2 (gallamine 20 nM; AFDX-116 1 pM) muscarinic antagonists. The neostigmine-induced hyperpolarization was associated with a decrease in input conductance and appeared to reverse near the potassium equilibrium potential. These results suggest that neostigmine may activate m2 receptors on cardiac parasympathetic postganglionic neurons, independent of its anticholinesterase activity. Supported by NIH grant NS 2978 to R.L.P.

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Among the deficits seen in Alzheimer's Disease are decreased levels of acetylcholine (ACh) in several brain regions. Attempts to remedy this deficit using either cholinergic or cholinesterase inhibitors have had very limited success. Direct pharmacotherapy has been plagued by the lack of selectivity of available drugs in discriminating among the five muscarinic receptors (m1-m5) that have been cloned to date. All of these receptors are coupled to the G-Protein superfamily with the m1 and m3 receptors linked to PI hydrolysis, and the m2 and m4 receptors negatively coupled to adenylyl cyclase (AC). Localization studies indicate that the m2 receptors are present in both salivary and sweat glands and brain. In the brain, m2 receptors are found predominantly postsynaptically, while the m1 receptors are found postsynaptically. This pattern has suggested that an ideal Alzheimer's drug might be a m2-selective, salivary and sweat gland, and brain. In the brain, m2 receptors are found predominantly postsynaptically, while the m1 receptors are found postsynaptically. These results suggest that neostigmine may activate m2 receptors on cardiac parasympathetic postganglionic neurons, independent of its anticholinesterase activity. Supported by NIH grant NS 2978 to R.L.P.

801.10 IN VIVO EFFECTIVENESS OF SELECT M1 ANTAGONISTS. M.J. Stillman*, B. Shuklit-Hale, A. Levy*, and H.B. Lieberman.
Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, "GEO-CENTERS, INC., Netwon, MA 02159, and TIRR, Nesa Ziona, ISRAEL.

The central cholinergic system has been shown to exhibit presynaptic modulation of acetylcholine (ACh) release. Although still under investigation, evidence from physiological and pharmacological studies suggests that the M1 receptor subtype acts as a negative autoreceptor. This in vivo physiological study examines the effects of the M3 antagonists, methoctramine, AF-DX 116, AF-DX 384, and AQA 741, on hippocampal extracellular ACh levels. Drug (2, 4, 8, or 16 mg) or vehicle (Ringer's solution) was perfused via a microdialysis probe into the rat CA1 hippocampus. Subsequently, freely-moving moving field fisher 344 rats. Levels of ACh were assessed by HPLC-EC. All compounds significantly elevated ACh levels. When the dose-response functions were expressed as Kd multiples, the drugs were found to be on the same curve. These results suggest that the concept that the proposed presynaptic mechanism of action of these drugs is M1 receptor-based.
ACETYLCHOLINE RECEPTOR MUSCARINIC: AGONIST/ANTAGONIST FOR RECEPTORS


According to pharmacological theory (Paton, 1961) even antagonists should cause receptor activation, initially, before tight binding occurs. Hart et al. (1993) reported on just such an agonistic effect of atropine (At) on muscarinic receptors of cardiac myocytes. The present observation occurred when At was used in an attempt to identify the receptor involved in an effect of acetylcholine (ACh) on sensory cells (hair cells) isolated from the frog sacule. The application of At (mM) produced changes in currents similar to those produced by ACh in voltage-clamped isolated hair cells. Further, ACh and At seemed to act additively. We then went on to determine that At had agonistic effects in vestibular organs in vitro. In both semicircular canal and saccule, At (pM) facilitated afferent firing rates similarly to ACh.

801.13 ANTICHOLERGIC ANTIPARKINSON DRUGS ARE POTENT ANTAGONISTS OF MUSCARINIC INHIBITION OF DI-STIMULATED ADENYLYL CYCLASE IN RAT STRIATUM. P.Ghelli and C. Oliai, Dept. of Neurosciences, Univ. of Cagliari, Cagliari, Italy.

Antimuscarinic drugs have long been used for the treatment of Parkinson’s disease and extrapyramidal motor disturbances caused by neurological disorders. However, the mechanisms of action and benefit of these drugs have not been elucidated. Previously, we have reported that in rat striatum muscarinic receptor activation inhibits dopamine (DA) D1 receptor function, as indicated by the reduced DA stimulation of adenylyl cyclase. In the present study we have investigated the ability of various antimuscarinic drugs currently used in the treatment of Parkinson's disease to antagonize the carbamyl (CCh) inhibition of adenylyl cyclase activity stimulated by the selective DA D1 agonist (SKF 89298). We have found that trihexyphenidyl, benztropine, biperiden, piperoxane and enprofylline antagonists of CCh inhibitory effect with Ki values ranging from 4.0 to 30 nM. Moreover, there is a good correlation (r = 0.95) between the Ki values and the average clinical doses of the drugs. These data suggest that the removal of a muscarinic inhibitory tone on D1 receptor activity may be one of the mechanisms by which the anticholinergic drugs exert their antiparkinsonian effect.

801.14 STRUTURAL BASIS OF ANTAGONIST BINDING TO m1 AND m2 MUSCARINIC SE-CEPTORS USING SITE-DIRECTED MUTAGENESIS. M.A.B. TICE*, L.A. TAYLOR, T. HASHMHI AND R.D. MCGUADT, CNS Pharmacology, Schering-Plough Research Institute, Kenilworth, NJ 07033.

Pharmacological characterization of the muscarinic receptors has been based on affinity for the M1 and M2 selective antagonists, pirenzepine (PZ) and AFDX-116, respectively. The molecular cloning and sequencing of the five muscarinic receptor genes have advanced our ability to investigate receptor binding of such tools as site-directed mutagenesis. In an attempt to map antagonist binding and to probe receptor selectivity, we have performed site-directed mutagenesis on the human m1 and m2 receptor genes. Corresponding single-base pairs were exchanged between the receptor genes. Mutants were constructed that replaced unchanged residues in m1 (Lox74-Ala175-Gly789-Ala177) with those corresponding charged residues of the m2 sequence (Glu172-Asp173-Glu174-Glu175). Another mutant involved the replacement of the charged Glu807 in m1 with Asn of m2. A similar mutant was constructed in m2 which changed Asn409 to Glu. Radioligand binding studies conducted with these mutants show 2 to 3 fold increase in affinity for PZ by the m2 mutants and a 2 fold increase in affinity of the m1 mutant Asn 367 for the m2 selective antagonist, hiribamicine.

Site-directed mutagenesis studies performed by Fraser et al. Mol. Pharm. 36:840(1989) on conserved aspartate residues of the m1 receptor revealed the importance of Asp105 for antagonist binding. We like others have investigated the effect of mutation of conserved amino acids around this pivotal residue. Mutation of the conserved Trp residue in m1 and m2 at positions 101 and 99, respectively, have been constructed. Construction of mutants in the muscarinic receptor family have given insight into the basis of receptor selectivity for various antagonists.


We recently reported that the apparent densities of m2/m3-like [3H]AFDAX-384 binding sites are not decreased, but increased, in various cortical and hippocampal areas of 24-25 month-old memory-impaired (AI) vs aged memory-unimpaired (AU) Long Evans rats behaviors-limited aged using the Morris Water Maze (Quirion et al., J. Neurosci., 15 1455, 1995; Aubert et al., Neuroscience, in press). Increases in M2-like receptor binding densities were correlated with altered acetylcholine release, and behavioral improvements were noted following treatment with a potent agonist, BEBN09 (Quirion et al., ibid). In order to determine if changes in M2-like receptor levels were genomic/transcriptional nature, the expression of molecularly-defined m1, m2, m3 and m4 receptor mRNAs was studied in the AI/AU model using quantitative in situ hybridization and "S"- labeled riboprobes complementary to human muscarinic receptors (DNA sequences [Bennet et al., Science 237:527-532, 1987]. Specific mRNA transcripts for each of these four muscarinic receptors are expressed in the rat brain according to a unique profile of distribution globally, but not fully, in accordance with earlier results obtained with oligonucleotides. The apparent levels of expression of each mRNA transcript was not significantly different in any regions of the AI vs AU rat brains or between 6-month old Long Evans adult rats and 24-25 month old aged animals. Accordingly, increases in [3H]AFDAX-384 binding sites are not associated with changes in the expression of the mRNA and transcripts, the two genes coding for pharmacologically-defined m2 receptors recognized by [3H]AFDAX-384. It thus suggests possible recycling of m2 receptors leading to apparent increases in receptor protein levels in the AI group. Supported by MBCC.


I-123 QNB, a radioligand agonist for muscarinic receptor imaging, has four stereoisomers which have not yet been fully characterized in vivo in humans. Two normal subjects (aged 34 and 75), and one normal SPECT studies with the RS, RR, SR and SS stereoisomers of I-123 QNB in separate scanning sessions. Prolonged timeactivity data over a period of 2 hours were obtained. Regions of Interest (ROIs) were drawn on brain SPECT scans. All isomers produced scans with no detectable binding in the cerebellum. The RS isomer produced scans with the most rapid accumulation of specific binding. The RR isomer produced scans with a slower accumulation and lower peak of specific binding. The RS and SS isomers yielded scans with an early peak of few counts and little anatomic aggregation of ligand distribution. The table displays the peak binding for both subjects in cpm/mcR.
082.3 SUBTYPE-SPECIFIC REGULATION OF MUSCARINIC RECEPTOR EXPRESSION AND FUNCTION BY HETEROMORPHIC RECEPTOR ACTIVATION. D. A. Jackson and N. M. Nathanson, Department of Pharmacology, University of Washington, Seattle, Washington 98195.

Isolated embryonic chick heart cells with the β-adrenergic agonist isoproterenol resulted in a dose-dependent increase in the level of mAChR 4 mRNA in the heart cells with the β-adrenergic agonist isoproterenol exhibited a greater increase in mAChR 4 mRNA than wild-type cells. The differences were most pronounced in the presence of cAMP, an inhibitor of cAMP-dependent protein kinase, which blocked the increase in the level of mAChR 4 mRNA.


Subtype specific muscarinic acetylcholine receptors (mAChRs) have been identified pharmacologically in human microvascular (MV) and capillary (C) cells, of which the M3 subtype is thought to mediate relaxation (Linville & Hamel, Naunyn-Schmied Arch Pharmacol., in press). We investigated the expression of mAChR mRNA by reverse transcription-polymerase chain reaction (RT/PCR) and in situ hybridization in human brain vascular endothelial cells. The results were obtained from primary cultures of normal human brain MVs and CAs obtained from postmortem brains of patients who had died of intracerebral hemorrhage. The expression of mAChR mRNA was determined by real-time quantitative RT/PCR using primers that amplify a 151-bp fragment of the M3 receptor cDNA. In situ hybridization was performed using a sense and antisense 32P-labeled RNA probe that hybridizes to the M3 receptor cDNA. The results showed that the expression of mAChR mRNA was higher in MVs than in CAs.

082.6 OXIDATIVE BURST IN NEURONAL CELLS AFTER MUSCARINIC STIMULATION. J. Naauli, J. Teto, J. Lottkenann, and K. Savolainen. National Public Health Institute, Department of Toxicology, P.O. Box 95, FI-70701 Kuopio, Finland, and Department of Environmental Sciences, University of Kuopio, Finland.

The effects of muscarinic receptor agonists, carbachol (CCh), on the production of reactive oxygen metabolites (ROMs) and on the levels of the intracellular glucose (GSH) were studied in human SH-SYSY neuroblastoma cell line. ROMs and GSH were measured by using fluorescent probes, dichlorofluorescin (DCF) and monochlorofluorescin (MCF), respectively. The concentration of intracellular protein kinase C (PKC) in the cell membrane was measured by photonel dihydrate (PDBu) binding and muscarinic receptor number was measured by using quinuclidinylbenzilate (QNB) binding to intact SH-SYSY cells. The intracellular GSH concentration increased upon stimulation of the cell with PDBu (1.8 fold at 60 min, 2.37 fold at 120 min, and 3 fold at 180 min as compared to the control values. CCh, at a concentration of 500 μM, caused a slight increase in ROM production, and values obtained with 100 μM CCh remained at control levels. Intracellular GSH levels decreased by about 25% when the cells were incubated for 120 min with 1 mM CCh. However, CCh levels returned back to the control level after 180 min incubation. PDBu binding showed by 73% after 20 min incubation with 1 mM CCh as compared to the control cells. Also 500 μM CCh increased in PDBu binding 33.54% as compared to corresponding controls, i.e. PKC translocation to the membrane was enhanced. QNB binding increased by 166% after 60 min incubation with 1 mM CCh, and returned back to the control level after 180 min incubation. We conclude that muscarinic receptor stimulation with CCh may cause oxidative stress in human neuroblastoma cells. Oxidative burst in neuronal cells may be partially through PKC, because increase in PDBu binding is a prerequisite for the production of ROM. However, there may be other effector mechanisms that have to be activated prior to the production of ROM. Supported by the Academy of Finland.


Current evidence suggests that neuropathies are a common complication of insulin-dependent diabetes mellitus (IDDM). Although structural and functional changes occur in neural tissues in individuals with diabetes, the molecular mechanisms responsible for these changes in neural function are not well understood. The BB diabetic rat is an in situ model in which to study these mechanisms since the BB rat model closely resembles human IDDM. The BB diabetic rat exhibits hyperglycemia and hyperinsulinemia (mChRs) in the major receptor for acetylcholine in the brain and its expression has shown to be affected by diabetes. Therefore, the neuronal mChR pathway in diabetes may be most relevant in diabetes. To investigate the expression of the mChR and downstream targets in diabetes in order to begin to dissect the role of altered mChR function in the development of metabolic neuropathies, the PRK activation by isoproterenol was performed on brain tissue homogenates from male diabetic BB rat vs. age- and sex-matched control rats. Diabetic rats were treated with insulin up to 30 min after the injection. The data demonstrate a two-fold increase in expression of mChR in diabetic rat brain. This change is specific for mChR expression and not downstream components of the signaling system (Gq, Gi, Gs, Go, G12, Go). In contrast, to laboratories channel (α2 subunit) expression is altered in the hippocampus, cortex, brainstem, or cerebellum of diabetic rats. Supported by a grant from NIH (NINDS) #31575-01.

082.8 ACETYLCHOLINE (ACh) STIMULATES THE RELEASE OF NITRIC OXIDE FROM RAT Speriences to spectroscopy. Z. Xu, C. Tong, J. C. Eisenach, and P. L. The Bowman Gray School of Medicine at Wake Forest University, Winston-Salem, NC 27110-1009.

Previous in vivo studies suggest that Nitric Oxide (NO) mediates the actions of ACh on spinal sympathetic neuronal activity and on anticoagulation in the spinal cord dorsal horn. In this study we utilized a novel biosensor for NO release to examine the effect of ACh perfusion of rat spinal cord in vitro on NO release. Following Animal Care Committee approval, adult male Sprague-Dawley rats were euthanized, the spinal cord removed, and spinal cord segments placed in tissue chambers perfused continuously with oxygenated Krebs solution which exited the chamber to fall on endothelium-denuded aortic rings. Successful removal of endothelium was tested by lack of relaxation to ACh. After preconditioning with phenylephrine, A (10−10 to 10−5 M) alone or with other drugs (all 10−4 M) were added into the perfusion solution, with continuous recording of the dilating ring’s tension. Data were expressed as % relaxation (mean ± SEM), and were analyzed by 2-way ANOVA on the full dose response curves, with p < 0.05 considered significant. Perfusion directly on receptor rings of ACh caused no change in their tension, while rinsing over rat spinal cord tissue with ACh resulted in a dose-dependent relaxation of the receptor rings, with maximum relaxation of 55%. This relaxation was blocked by BAPTA (10−4 M), hemoglobin, or nitroblue blue. ACh-induced relaxation was also blocked similarly by the muscarinic antagonist, atropine, prazosin, or APF161. This study showed that ACh causes release of a vasodilator from spinal cord slices in vitro which shares the pharmacology of NO (activescendin-endothelium-denuded rings, blocked by the NO synthase inhibitor, L-NNA, the NO scavenger, hemoglobin, and the glycylated cyclam inhibitor, methylene blue), and involving both M1 and M2 receptors activation. Supported in part by GM53523.
082.9

EFFECT OF FORSKOLIN ON CARBACHOL-INDUCED SIGNAL TRANSDUCTION IN CANINE CULTURED TRACHEAL SMOOTH MUSCLE CELLS. C.M. Yang*, Department of Pharmacology, Chang Gung College of Medicine and Technology, Keelung-Taoyuan, Taiwan.

The effect of elevating cyclic AMP on carbachol-induced generation of inositol phosphates (IPs) and rise in intracellular Ca2+ ([Ca2+]i) was investigated in canine cultured tracheal smooth muscle cells (TSMCs). Pretreatment of TSMCs with either cholera toxin, forskolin, or diethylcarbamyl AMP inhibited carbachol-stimulated Ca2+ mobilization and IPs accumulation. The inhibitory effects of these agents produced both depression of the maximal response and a shift to the right of the concentration-response curve of carbachol without changing the EC50 values. Even after treatment with forskolin for 24 h, the cells retained the ability to respond to carbachol-induced Ca2+ mobilization to the same extent as the control group. The Kd and Bmax values of the muscarinic receptor (mACr) for [3H]-NMS scopolamine binding were not significantly changed by forskolin treatment, suggesting that the inhibitory effect of forskolin in basal mACr. The A2F-induced IPs accumulation was inhibited by forskolin, suggesting that G protein(s) are directly activated by A2F, and uncoupled to phospholipase C by forskolin treatment. We conclude that cyclic AMP elevating agents might contribute to the inhibitory effect of cyclic AMP on tracheal smooth muscle function.

082.11


The present study determined the effect of (3)-epitapidine (Epi), a neuronal acetylcholine receptor (NACHR) agonist on catecholamine release. (3)-Epi (3-300 nM) produced a concentration-dependent increase in [3H]-dopamine release from rat striatal slices, and [3H]-norepinephrine release from rat hippocampal and thalamic slices, with differential sensitivity to various NACHR antagonists as shown below:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>EC50 (nM)</th>
<th>Mec (3 mg/kg, s.c.)</th>
<th>d-TC (100 mg/kg, s.c.)</th>
<th>EHIE (100 mg/kg, s.c.)</th>
<th>TTX (1000 mg/kg, s.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatum</td>
<td>37 ± 10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>29 ± 2.6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thalamus</td>
<td>23 ± 5.3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

In addition, (3)-Epi (1.3 mg/kg, s.c.) and nicotine (200 mg/kg, s.c.) produced ipsilateral turning in the unilaterally 6-hydroxy-dopamine lesioned rat, consistent with the stimulation of dopamine release from the intact nigrostriatal dopamine pathway. This effect was blocked by mecamylamine (mec) (3 mg/kg, s.c.). Moreover, (3)-Epi (1-3 mg/kg, s.c.) increased locomotor activity in a dose-dependent manner. This effect was blocked by mec as well as the D1 and D2 receptor antagonists, SCH23390 and eticlopride, respectively. These results demonstrate that, in addition to the previously reported anergic activity, (3)-Epi displays NACHR agonist activity in the rat central nervous system and that certain effects may be mediated via NACHR-stimulated catecholamine release, and subsequent activation of corresponding receptors.

082.12


In the absence of ligands, G-protein coupled receptors interconvert between active and inactive conformations. These conformations are stabilized by agonists and antagonists, respectively. Like agonists, G-proteins are thought to preferentially associate with receptors in the active conformation and should therefore be able to promote their formation in the absence of agonist. We show that overexpression of Q2b, but not Q2a induces constitutive activation of comparable (m1, m3, and m5 but not m2) muscarinic receptors and this activity is blocked by muscarinic antagonists. Q2a also increases the potency and efficacy of agonists. The phenotypes of receptors activated by G-proteins are very similar to the phenotypes of mutationally activated receptors. These results indicate that regulation of G-protein levels has a profound impact on receptor control of cellular physiology, even in the absence of agonist ligands.

082.13

G PROTEIN f3 SUBUNITs IMIC THE MUSCARINIC REGULATION OF ADENYLYL CYCLASE OF RAT OLFACTOR Y BULB. M.C. Oliva*, H. Hamer*, and P. Oliosi*, Dept. of Neurosciences, University of Cagliari, Italy and Dept. of Biophys. and Biophys., Univ. of Chicago, IL 60680.

In membranes of rat olfactory bulb washout of muscarinic receptors enhances basal and neurotransmitter-stimulated adenyl cyclase activities but inhibits Ca2+/calmodulin and forskolin (FSK) stimulations of the enzyme. In the present study we show that this bimodal control of cyclic AMP formation can be reproduced by the G protein f3 subunits. Thus, incubation of olfactory bulb membranes with the f3 subunits of transducin elicited a concentration-dependent increase of basal adenyl cyclase activity with an EC50 of about 100 nM. The f3 subunit effect was not additive with that produced by maximal activation of muscarinic receptors. Moreover, as observed with muscarinic receptor agonists, the f3 subunits significantly potentiated the stimulation of cyclic AMP formation by vasopressin intestinal peptide and inhibited the adenyl cyclase activity stimulated by FSK with an IC50 of about 40 nM. These data support the idea that in rat olfactory bulb muscarinic receptors exert a bimodal control on cyclic AMP formation by promoting the release of G protein f3 subunits which then differentially affect the various molecular forms of adenyl cyclase.
803.1 HYDROGEN PEROXIDE AND HYDROXY RADICAL, BUT NOT SUPEROXIDE, PROMOTE A CONCENTRATION-DEPENDENT INHIBITION OF GLUTAMATE UPTAKE BY ASTROCYTES. O. Sorg and F.E. Bloom. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Glutamate uptake by astrocytes is a very important function which prevents the potential neurotoxicity of this neurotransmitter after its release by neurons. Recently, Volkert et al. showed that H2O2 induces an inhibition of glutamate uptake by primary cultures in vitro. Using the same model, we tried to define the role of three main reactive oxygen species in Glu uptake inhibition, as well as the efficiency of endogenous protective mechanisms by inhibition of a Cu2+ up in a concentration-dependent manner. Cu2+ by itself, has no effect, suggesting that the reactive species in the xanthine/xanthine oxidase system is a Cu2+ dependent reaction produced by the Haber-Weiss reaction. The Glu uptake inhibitor effect of H2O2 is potentiated by adding CuSO4 and ascorbate - a condition known to produce OH radical - or N-ethylmaleimide. The latter compound could act as a thiol-modifier group of the glutamate transporter and/or as a glutathione peroxidase I inhibitor; these possibilities are under investigation. (Supported by MH 47688).


Diverse metabolic insults can cause neuronal damage via the release of excitatory amino acids (EAAs), including glutamate (Glu). Severe ATP depletion releases EAA's by reversal of co-transporters that normally clear the synapse of transmitter EAAs. However, many metabolic insults produce only mild to moderate ATP depletion. Therefore, the effects of moderate ATP depletion on EAAs were studied. Using an EAA analog D,L-threo-hydroxyaspartate (OH-Asp) was used as an indicator of Glu uptake. OH-Asp uptake and the ATP content of hippocampal slices were measured by HPLC. Moderate ATP depletion (80% > ATP > 60% of control) increased uptake of OH-Asp. In contrast, more severe ATP depletion (ATP<40%) caused a decrease OH-Asp uptake and increase of endogenous Glu, suggesting reversal of co-transporters.

Selective depletion of ATP from glia using fluorocitrate and fluorouracil resulted in a similar increase in uptake of OH-Asp, suggesting that much of the increased uptake occurred in glial cells. Immunocytochemical localization of Glu in metabolically insulted slices indicated that Glu was decreased in nerve terminal-like paxons and increased in cells with glial-like morphology, suggesting that glial cells accumulate EAAs released by neurons. Increasing extracellular phosphate blocked the increased uptake of EAAs, suggesting that the decreases of intracellular phosphate by ATP may increase the phosphate gradient and potentiate EAA uptake. The increased uptake of EAAs during mild to moderate ATP depletion suggests that during many moderate insults, such as partial ischemia, a decrease in extracellular Glu might contribute to loss of CNS function.

803.3 GLUTAMATE UPTAKE BY HIPPOCAMPAL ASTROCYTES IS INHIBITED BY FERROUS CHLORIDE. J. H. Pirzonia*, E. O'Connor and D.L. Kramer. Dept. of Surgery, (Neurosurgery), Yale University School of Medicine, New Haven, CT 06520.

Iron-complexed to blood breakdown products has been implicated in the pathogenesis of stroke and reperfusion injury, trauma and epilepsy. Excessive accumulation of iron during aging has also been implicated in several neurodegenerative diseases. We hypothesized that iron may affect neuronal injury by inhibiting glutamate transport mechanisms. As a partial model of glutamate is primarily responsible for modulated synaptic glutamate activity, we examined the effect of iron on glutamate uptake and efflux in astrocytes. Hippocampal astrocytes were prepared from neonatal rats and preincubated in HEPES buffer containing 50 μM ferrous chloride for 30 minutes before the addition of H3-glutamate. Uptake was determined from 2-60 minutes and total radioactivity determined from cell lysates. Ferrous chloride decreased glutamate uptake by 50% at 10 minutes (n=4, p<0.05) and by 70% after 60 minutes (n=4, p<0.001) compared to control cells incubated in HEPES buffer without iron. In separate experiments the rate of H3-glutamate efflux was not changed by addition of 50 μM ferrous chloride. Precipitation with the calcium channel blocker, lanthanum chloride, (1 μM) did prevent inhibition of H3-glutamate uptake by 50 μM ferrous chloride. We conclude that iron inhibits glutamate uptake into astrocytes and thereby may induce neurotoxicity by a mechanism independent of transmembrane calcium influx.


Uptake in neurons and astrocytes by distinct transporter subtypes is low (<1 μM) extracellular glutamate concentration (EGlu), with crucial implications for excitotoxic synaptic transmission. In neurons, loss of the physiological icGluD receptor decreases transporters function in reversed mode pumping out Glu up to neurotoxic levels. We have reported that addition of a competitive Glu uptake inhibitor, 3- pyrrolidine-2,4-dicarboxylate (PDC, 200 μM, >80% inhibition) in rat hippocampal preparations, induces rapid increase of cell-released Glu (EGlu, 10 nM to 5 μM, and, as a consequence, delayed (1 day) neurotoxicity (Volkert et al., Soc.Neurosci. Abstr. 1992;1994). Today it is prevented by the NMDA-antagonist APV (100 μM) and attenuated (40-60%) by AMPA/kainate (CNQX, 10 μM) and metabotropic (MCPG, 500 μM) blockers. Surprisingly, both (GLU), increase and neurotoxicity are largely (70%) unaffected by 1 M TTX, despite that TTX abolishes excitatory synaptic activity in the culture, as recorded by whole-cell current-clamped neurons. However, in the presence of TTX, we observe GLU release from astrocytes of sister glial cultures in response to PDC (200 μM, >80% inhibition) by >80% the TTX-insensitive component of (GLU), increase observed in co-cultures. Therefore, our data indicate that: (1) transporter-mediated (Li+-sensitive) GLU efflux from both glia and neurons, in addition to reduced uptake of synaptically released GLU, accounts for PDC-induced GLU, rise and neurotoxicity; (2) reversed GLU transport function can be activated even under normal, non-ischemic conditions (e.g. via heteroxechange) with direct neuroexcitotoxic consequences.

803.5 HUMAN NERVE TISSUE-SPECIFIC GLUTAMATE DEHYDROGENASE ADAPTED TO FUNCTION UNDER LOW ENERGY STATES AND INCREASED GLUTAMATE RELEASE. P. Shabat & R.B. Donald.

Human glutamate dehydrogenase (GDH) exists in nerve tissue-specific and housekeeping isoforms encoded by distinct genes. We have obtained both these proteins in pure form by expressing the corresponding cDNAs in Sf9 cells using the baculovirus expression system. The specific expression of each GDH isoform by the corresponding cell lines was verified by the N-terminal amino acid sequences of the housekeeping and neuroglial GDHs. The neuroglial GDH was heat-labile and the housekeeping enzyme heat stable. In the absence of allosteric effectors, the two isoforms differed markedly in their ability to interconvert glutamate and ammonium. The neuroglial activity was largely inactive whereas the housekeeping GDH showed 40-50% of its maximal activity. ADP induced a concentration-dependent (10-1,000 μM) activation that was proportionally greater than the housekeeping GDH (50-260%). GTP, known to be present in brain at relatively high levels, inhibited the housekeeping GDH but had little effect on the nerve tissue-specific enzyme. Since GDH is highly concentrated in synaptic astrocytes where it is involved in transmitter glutamate metabolism, these allosteric properties clearly represent an adaptation enabling the brain-specific GDH to oxidize increased levels of neuronal glutamine in the face of decreased extracellular levels of ATP to ADP that may occur in energy failure and/or increased glutamate release.

803.6 REGULATION AND PATHOLOGY OF KYNURENINE PATHWAY METABOLISM IN RAT BRAIN. L. Lustenauer, Eva Vagnerova, Urban Tvedeleva and Bodil Enevoldsen.

The kynurenine pathway, which is the principal route of hepatic L-tryptophan (TRP) metabolism, also appears to play an important role in brain function. A kynurenine pathway metabolite of particular interest in this regard is quinolinic acid (QUIN), which acts as a NMDA receptor agonist and excitotoxin. Endogenous QUIN and its biosynthetic enzyme 3-hydroxyanthranilic acid deaminase (3-HAD) occur in the brain and QUIN levels have been shown to increase in various neurodegenerative processes. In cultures of rat tissue, exposure to QUIN and the precursor 3-hydroxykynurenine (3-HK) produced a similar loss. Furthermore, administration of QUIN or the precursor in vivo to rats leads to cerebral damage. However, the knowledge about the relative contribution of peripheral and central kynurenine pathway enzymes in the in vivo regulation of cerebral QUIN is limited. In Sprague-Dawley rats, microinjection of (i.c.v.) administration of 3-HANA, but not other precursors of QUIN, induced a major increase in hippocampal QUIN, that was saturable at high doses and peaked at 24 h after administration. Co-administration with a 3-HAD inhibitor blocked the elevation of hippocampal QUIN. Systemic administration of tryptophan, 3-HANA and QUIN itself increased cerebral QUIN, while much smaller effects were seen after administration of L-kynurenine and 3-hydroxykynurenine. Systemic, but not i.c.v., administration of 3-HAD inhibitor partially counteracted the increase of cerebral QUIN after systemic TRY or 3-HANA. However, in animals with brain damage, such as ischemia and neurotransmitter-induced lesions, i.c.v. administration of the inhibitor decreased cerebral QUIN. This indicates that, although cerebral QUIN levels are regulated by the availability of precursors in the periphery, cerebral synthesis plays a significant role when central QUIN levels are enhanced under pathological conditions.
803.7
INTRACEREBRAL GLUTAMATE AND CEREBRAL BLOOD FLOW DURING CHRONIC HYPOXIA IN THE NEAR-TERM OVINE FETUS.
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University of Iowa College of Medicine, Iowa City, Iowa.

Using chronic in utero microdialysis (ref.), we examined the relationship between cerebral blood flow (CBF) and glutamate (Glu) efflux from the parasaggittal parasagittal cortex (PSPC) in the near-term ovine fetus during chronic fetal hypoxia. Six near-term ovine fetuses (2 control, 4 experimental) were chronically instrumented with microdialysis catheters and a microdialysis probe inserted in the PSPC. An adjustable vascular occluder was placed around the common uterine artery. After a 3 day recovery and a 24 hour probe equilibration period the vascular occluder was tightened in experimental animals to reduce the fetal O2 saturation by 50% for a 24 hour period without progressive metabolic acidemia. Fetal CBF was determined by injection of radiolabeled microspheres with 8, 16 and 24 hours. Microdialysate samples were collected at 28 minute intervals and the concentration of Glu determined by HPLC. Basal Glu efflux was 102 ± 64 nmol/ml and basal CBF was 207 ± 61 ml/gm ± min. In 3 of 4 experimental animals there was an increase in CBF and Glu efflux in the PSPC while in 1 experimental animal both controls the CBF and Glu were unchanged. All fetuses experienced an increase in Glu efflux post mortem.

In this preliminary study, ovine fetuses responding to hypoxemia by increasing CBF also show an increase in PSPC Glu efflux. Glu did not increase in animals that failed to show an increase in CBF.

Supported by NINDS 1R01NS34567-01 and Graver Clinician Scientist Award

803.8
LONG-TERM CHANGES IN BRAIN FOLLOWING CONTINUOUS PHENICYCLIDINE ADMINISTRATION: STUDIES USING 2-DOG, FLUNITRAZEPAM, KETAMINE, AMPA, AND LIGANDS FOR AMPA AND PCP RECEPTORS. G. D. Elliott* and A. KEV.
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When given continuously for several days, NMDA antagonists such as phenycyclidine (PCP) and dizocilpine (MK-801) induce neural degeneration in a variety of limbic structures such as retrosplinal cortex (RSOc), entorhinal cortex (ENTc), dentate of hippocampus (HPC), and olfactory areas. This has been demonstrated using a variety of measures, including silver-stains. Further autoradiographic studies were conducted in animals at both 24 hours and 21 days after binge PCP administration using 2-DOG and a variety of receptor ligands.

24 hours after pellet removal there were still large increases in glucose uptake in these same limbic structures, and many of these regions were still present after 21 days of recovery. These alterations were accompanied by decreased flunitrazepam but increased mazindol binding in many of these same limbic regions. ORS and AMPA binding was decreased but TCP binding increased in many brain regions, including striatum. These studies may indicate an anatomical substrate for the persisting psychosis which sometimes occur following PCP.

803.9
HIPPOCAMPAL EXCITATION AND TOXICITY PRODUCED IN VIVO BY DOMOIC ACID. P.M. Grupp*, J.M. Polichack*, D.S. Watamaniuk, D.P. Weaver.*
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It is well documented that domoic acid (DOM), a natural product of the dinoflagellate Nostocales, is a neurotoxin to animals and to humans. In an attempt to determine the mechanism of toxicity, rats were injected IP with DOM (25-100 pg/kg) and used to microinjected DOM into the parasagittal parasagittal cortex of rats in order to study the effects of the lesion on the rat hippocampus. The results of this study were compared to the findings of the same model with a microinjection of glutamate (Glu).

803.10

Domoic acid (DOM) is an excitatory amino acid analog that has been identified as a potent neurotoxin in both people and experimental animals. The effects of DOM in the mature brain have been well documented but little information is available concerning its action in the immature brain. This study was designed to assess the toxic effects of systemically administered domoic acid in developing male and female Sprague-Dawley rats. Intraperitoneal injections of doses (n=6 for each) of DOM or saline were administered on postnatal days 0, 5, 14, 22, 30 (f) and 50 (m). Dose response curves as a function of age were generated and statistically compared for parallelism and potency. Prior to warning pups were found to be significantly more susceptible to domoic acid induced toxicity, as indicated by both increased potency and the occurrence of convulsive behaviours involving both fore and hind limbs. Post-weaning the more classic limbic seizure was observed and was characterized by the occurrence of forelimb tremors in a reared position and masticatory movements. Data indicate that susceptibility to domoic acid toxicity in the developing rat may be dependent upon establishment and maturation of limbic system circuitry.

803.11
Pathology Section, Toxicology Research Div., Bur. of Chemical Safety; Food Div., HBP, Ottawa, Ont., Can. K1A 0L2.

Annexic shellfish poisoning (ASP) is caused by eating shellfish containing elevated levels of domoic acid, which is a harmful algal bloom. This study examined the effects of repeated consumption of DA. Male and female Sprague-Dawley rats were dosed by gavage with 64 days with 0, 0.1 or 5 mg/kg/day of DA. Treated animals showed behavioral changes over the 64 days, including urinolysis, haematoxylin and gentian blue staining. At the end of the study and under anesthesia, rats were exsanguinated and perfused with neutral buffered formalin (NBF) with 2% glutaraldehyde (GA); 2% paraformaldehyde (PF). Tissues were then processed for light microscopy (LM) or for electron microscopy (EM). LM results were unremarkable, including the area postrema, hippocampus and retina, which are considered target sites for DA toxicity. Glial fibrillary acid protein (GFAP) and gluta- mase receptor immunohistochemistry (GluR) did not show visually detectable differences between treated and control rats. EM of the CA3 field of the hippocampus from the high dose (5mg/kg/day) group revealed neuronal damage: cytoplastic vacuolation, neuronal shrinkage, dilatation of dendritic and astrocytic processes and formation of electron dense protrusions of the damaged axon. The damaged axon was demonstrated to the estimated maximum human dose during the Canadian ASP incident in 1987 and is seven times less than that required to cause overt clinical signs in the rat. No significant changes were observed at the dose of 0.1 mg/kg, estimated as the dose resulting from the consumption of one 250 mg portion of mussel meat containing the present limit for DA of 20μg/kg of shellfish meat.

Supported by the Canadian MRC.

803.12
SERUM CLEARANCE OF DOMOIC ACID IS UNALTERED FOLLOWING MULTIPLE EXPOSURES IN MICE. Y.G. Peep, H.F. Martin* and L. Barnadiego, Marine Biotoxins Program, National Marine Fisheries Service and Marine Biological & Environmental Sciences, Medical University of South Carolina, Charleston, SC 29412.

Domoic acid (DA), a bicyclic amino acid, is a rigid analogue of the neurotransmitter L-glutamate that has been implicated as an environmental neurotoxin to humans. Previous studies in rodents and primates indicate that DA is cleared from serum within four hours after a single dose. In the present study, we have determined the concentration of DA in serum after single or multiple dose exposure. To correlate this with stereotypic neurological effects in mice. Mice were intraperitoneally administered in single or multiple doses (every other day i.p. for 4 times in 7 days) or a single dose on the same day of the last multiple exposure dose. DA levels were monitored as serum concentration and measured at 60 min after each dose using the DA radio receptor assay. Serum DA levels did not differ at 60 min in single (0.59 ± 0.04 μg/ml) and multiple (0.50 ± 0.05 μg/ml) exposures, or at 120 min (single 0.124 ± 0.005 μg/ml, multiple 0.123 ± 0.004 μg/ml, respectively n= 7). The onset of stereotypic neurological effects in the form of scratching was also similar in each group (20.2 ± 0.6 min & 22.4 ± 1.1 min, respectively) although the duration of scratching lasted longer in mice with a single dose (30-45 min) than those with multiple exposure (20-35 min). Convulsive behavior, evident with a single 1 mg/kg dose, was not observed after the second dose in the multiple exposure group. This study indicates that multiple exposures of DA to mice does not alter DA clearance from the serum and does not appear to lead to a more neurotoxic response.
804.1 DISTRIBUTION OF GABA<sub>A</sub> RECEPTOR α1-SUBUNIT POLYPEPTIDES
IN THE GUINEA PIG HIPPOCAMPUS. E.M. Barnes, Jr.*, M.E. Diaz, I.V.
Colom, J.D. Miranda, B.J. Baumgartner, and M.H.J. Tothomi, Depts.
of Neurology and Biochemistry and Div. of Neuroscience, Baylor Col. of
Med., Houston, TX 77030.

In order to conduct a histochemical examination of GABA<sub>A</sub>
receptors in various brain subregions, we have prepared polyclonal
antibodies against a selective intracellular loop region of the α1 subunit.
The chick GABA<sub>A</sub> receptor α1(331–381) subunit sequence was
expressed as a fusion protein containing a histidine tag leader peptide,
purified by Ni<sup>2+</sup>-affinity chromatography, and used for rabbit
immunizations. The corresponding and subunit antisera immunoprecipitated 66% of H-flunitrazepam binding to extracts from guinea pig hippocampus and reacted with a single 50-kDa polypeptide on Western blots. The strong cross-species reactivity was expected from the 88% sequence identity in the α1(331–381) region between chicken and rodents. The pattern of immunoreactivity on 50 μm sections of guinea pig hippocampus was examined using rhodamine-labeled anti-rabbit
IgG and confocal laser microscopy. Immunohistochemical labeling of GABA<sub>A</sub>
receptor α1 subunits was observed on pyramidal cells and putative
interneurons in the CA1 and CA3 regions. The α1 subunit was located in the
soma and apical dendrites of pyramidal cells. This distribution was similar
for the both the CA1 and CA3 layers. Putative interneurons of the stratum oriens,
pyramidal, and radiatum also showed immunoreactivity of α1 subunits.

Supported by DK17436, MH4715, GM14156, and NS11535 from NIB.

804.3 DISTRIBUTION OF GABA<sub>/</sub> Benzodiazepine-RECEPTORS IN
THE BRAIN OF GOLD FISH (CARASSIUS AURATUS) AND
of Zoology, University of Lund, Sweden.

The antibody bi-d7 was used for immunocytochemical detection of
GABA<sub>A</sub>/benzodiazepine receptor β2/3-subunits (bd-17r) in the brain
cell bodies of the goldfish brain. In the brain cell bodies of the goldfish brain, bd-17r were analyzed by computerized image analysis and were compared
with our previous results from the salmon. In the goldfish bi-d7r is mostly found as a diffuse labeling of circumcorted areas corresponding
with cytoarchitectonic defined "nuclei". Striatum is observed in the telencephalon, pretectum, optic tectum, hypothalamus and torus semicircularis. Several nuclei of the hypothalamus and posterior tuberculum show strong labeling, (e.g., the preganglular nucleus, n.
terior tuberis, n. posterior tuberis, lobis infereories). The striatum
periventricular, striatum griseum centrale and striatum fibrosus et
griseum superficiales of the optic tectum display bi-d7r. Perikaryal
labeling was observed in the torus longitudinalis and in the granular
layer of different subdivisions of the cerebellum. In the brain stem the
central grey is strongly immunoreactive. The bd-17r in the goldfish
brain is in general similar to that found in the salmon, but in the
goldfish labeling generally correlates better with cytoarchitectonic
tissues, particularly in the hypothalamus and posterior tuberculum.
The labeling in the central grey is not present in the salmon brain.

804.4 IMMUNOCYTOCHEMISTRY OF A NOVEL GABA
RECEPTOR SUBUNIT
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Madison, Wisconsin 53706.

Following our recent cloning of a novel γ-aminobutyric acid
(GABA) receptor subunit gene Resistance to δ-9-tetrahydrocannabinol is
encoded by a gene is located on chromosome 14. We have been
interested in defining its pattern of expression during development. Here we report the raising of an anti-R polyclonal antibody that recognizes a single protein of the expected 65 kDa size in immunoblots of DR1morphine homogenates. In situ hybridization with DR1/cDNA probes and the anti-R antibody shows that the R message and protein are expressed globally in the developing central nervous system (CNS) of 15-17 day embryos. No
message can be observed on or before 12-13 hr. Interestingly, despite the use of GABA in both the periphery and the CNS, all anti-R receptor subunits appear to be confined to the CNS. Detailed
immunocytochemistry of DR1morphine brain sections showed particularly
strong anti-R antibody staining in the optic lobes, ellipsoid body, fan
shaped body, ventralis lateralis protocerebrum and the glomeruli of the antennal
lobes. Results are compared with the distribution of staining observed in the
insect CNS with antibodies against GABA itself, synaptophysin (a
synaptic vesicle protein) and a second DR1morphine subunit which appears to
be a homolog of the vertebrate GABA<sub>A</sub> receptor β subclass.

804.6 DIFFERENTIAL DISTRIBUTION OF GABA<sub>A</sub> RECEPTOR SUBUNITS IN THE RAT
NEURAL MIDBRAIN CORTEX: RELEVANCE TO NOVEL ANTIPSYCHOTIC DRUG
TREATMENT. E. Dunn, J. H. Fischbein, D. B. Carter and K. M.
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49001 and Institute of Pharmacology, Univ. of Zurich.

The prefrontal cortex has been implicated in manifestation of negative
symptoms and cognitive deficits in schizophrenia patients. Several
studies of postmortem human brain tissue indicate that there is a loss of GABAergic interneurons and an increase in GABAergic receptor binding in the prefrontal
cortical regions of schizophrenics. These data suggest that disturbances
in GABAergic neurotransmission in limbic cortex of schizophrenic patients may contribute to the pathophysiology of schizophrenia, and that specific GABA-
eric agents may be therapeutically useful. To evaluate this possibility, we
have performed a detailed autoradiographic analysis of GABA<sub>A</sub>
receptor subunits in the rat medial prefrontal cortex (orbitofrontal, IL region). Mapping of α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, α<sub>4</sub>, and β<sub>3</sub> subunits carried out by immunocytochemistry
with characterized, subunit-specific antibodies. The α<sub>1</sub> and β<sub>3</sub> subunit antibodies immunostained all layers (I, II, III, V, VI) of the IL
cortex, whereas α<sub>2</sub> and α<sub>3</sub> displayed labeling that appeared to be limited to interneurons. In contrast, α<sub>4</sub> and β<sub>3</sub> immunoreactivity was restricted to a single layer of the IL region. The data suggest that these agents have different binding sites on the receptors, with α<sub>1</sub>-like sites on layer VI and α<sub>2</sub>-like sites on layer II and III. These findings suggest that these agents may have different therapeutic effects in schizophrenia and may be useful in targeting different populations of GABAergic neurons.
804.7

GABA, an inhibitory neurotransmitter, plays an important role in the nucleus tractus solitarius (NTS). The nucleus is associated with taste sensation, general visceral input and regulation of the autonomic nervous system. Previous immunohistochemical studies have demonstrated that GABA-positive terminals and neuronal somata are richly distributed in the NTS.

In this study, we examined the immunohistochemical localization of GABAergic receptors in the rat NTS using a monoclonal antibody against the 

The pattern of distribution was compared with that of presynaptic GABA terminals. Immunoreactivity for GABA receptors was confined to the surface of neuronal somata and processes. These positive structures were distributed mainly in the rostral part of the NTS, where GABA terminals were also abundant. In the intermediate and caudal parts of the NTS, however, positive staining for GABA receptors was located in the ventrolateral part of the NTS, particularly in the ventrolateral subnucleus. In these regions, GABA-positive terminals were rather sparse. In addition, few if any cells positive for GABA receptors but that their actions in the intermediate and caudal parts of the NTS appear to be mediated by receptors that are not detected by the monoclonal antibody used.

804.9
KINETIC PROPERTIES OF EVOKED MONOSYNAPTIC FAST IPSCS IN NEOCORtical PYRAMIDAL CELLS. D. S. Lin1 and L. B. Karceski. Deps. of Pharmacology and Neurology, SUNY-Health Science Center, Brooklyn, NY 11203.

Previously, we examined the recruitment properties of fast IPSCs in neocortical layer V pyramidal cells (PCs) and found that the magnitude of fast GABAergic inhibition is absolutely limited. However, overall inhibitory strength is also determined by IPSC kinetics. For example, if IPSC time decay is prolonged as a consequence of volume transmission, an increase in total charge transfer results. We have, therefore, examined the kinetic properties of fast IPSCs using whole-cell techniques to record from layer V PCs in slices (400μm) of somatosensory cortex from young (P15-30) rats. Fast IPSCs were evoked by graded stimuli applied to layer IV. Cation-selective electrode solutions were employed to block slow GABA-mediated IPSCs and excitatory synaptic transmission was blocked with CPP and CNQX (10μM). Isolated IPSCs were best fit by an equation describing second-order activation and single-exponential current decay. There was no apparent correlation between IPSC kinetics and stimulus intensity. When IPSCs reached maximal amplitude, further increases in stimulus intensity did not cause significant changes in either IPSC rise or decay. The consequences of these kinetic properties for limited fast inhibition and the underlying pre- and postsynaptic mechanisms will be discussed. (Supported by MHS1677)

804.11
GABAergic MODULATION OF NEURONS FROM THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (HDBB) THAT PROJECT TO THE HIPPOCAMPUS. J.G. Eawas, B.S. Jessar, K.H. Harris, and J.H. Jiangadas. Department of Medicine (Neurology) and Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada.

Anatomical and physiological studies have shown that the HDBB and the hippocampus. GABA has been identified as a major transmitter in these pathways, which have been implicated in the generation and maintenance of epilepsy in these animals. We have examined the actions of GABA receptor agonists on acutely, dissociated rat HDBB neurons using the whole-cell patch-clamp technique. Fluorescein-labeled latex microspheres were injected into the hippocampus to retrogradely label cells in the HDBB. Thereafter, a few days later, cells were acutely dissociated using enzymatic treatment and visualized under fluorescent microscopy to identify the labeled neurons. Current-voltage relationships of the HDBB neurons were similar to those recorded from unlabelled cells. Under voltage-clamp conditions, both applied muscimol (10μM), a GABA receptor agonist, evoked a current that reversed at -60 mV (n=4). Baclofen (10μM), a GABA receptor agonist, evoked no response (n=4). These results suggest that GABAergic afferents modulate the activity of the HDBB neurons projecting to the hippocampus through the GABA receptor.

Supported by the Medical Research Council of Canada, the H.M. Toupin Foundation and Alberta Heritage Foundation of Medical Research.

804.8
Ba²⁺-SENSITIVITY OF MONOSYNAPTIC AND 4-AP-INDUCED GABA, IPSPs IN CA1 PYRAMIDAL CELLS OF RAT HIPPOCAMPAL SLICES. T.M. Plaut1 and J.C. Lasek. Center for Research in Neurological Sciences and Department of Physiology, University of Montreal, Montreal, QC, Canada H3C 3J7.

GABAergic receptors in hippocampal pyramidal cells may be coupled to heterogeneous K⁺ conductances that are differentially sensitive to Ba²⁺. The aim of this study was to examine with intracellular recordings the effects of barium on GABA-mediated inhibitory post synaptic potentials (IPSPs), pharmacologically isolated in the presence of GABA, NMDA and recent-NMDA receptor antagonist, in conventional rat hippocampal slices. In a first series of experiments, monosynaptic GABA, IPSPs were evoked by stimulation of GABA fibers in ent. radiatum, lacunosum-molecular or oriens. These IPSPs displayed the following characteristics (mean ± SEM): -5±0.5 mV, peak latency 15±22 ms, and recovery time 38±37 ms (n=15). The peak of these IPSPs was 91±6.2±2 mV (n=12). Bath application of 1mM BaCl₂ completely suppressed monosynaptic GABA, IPSPs to -59±16.0±2 mV (control amplitude: -89±7±2; p<0.001). Ba²⁺ effects were reversible upon washout (42±0.19±4 of control amplitude; n=4). In a second series of experiments, spontaneous GABA, IPSPs were induced by bath application of 100 μM 4-aminopyridine (4-AP). Spontaneous 4-AP induced the following properties: amplitude: -8±1.6±2 mV, rise time 21±20 ms, recovery time 1±0±4±2 ms. The E<sub>rev</sub> was -107±2±6±3 mV (n=7). Synchronized GABA, IPSPs were progressively abolished by bath application of 4-AP (n=8). Spontaneous GABA, IPSPs recovered after washout of BaCl₂ (64±8±4±2 of control amplitude, n=2). These results indicate that GABA, IPSPs, either evoked monosynaptically or synchronously induced in 4-AP, were mediated by K⁺ conductances sensitive to Ba²⁺. Thus, synthetically released GABA may not activate post synaptic GABA receptors linked to Ba²⁺-sensitive K⁺ conductances.

[Supported by the MRC, FRQ, FCAR and SavoY Foundation]

804.10
DUAL MODULATION OF GABA RECEPTORS BY EXTERNAL H⁺ IONS IN ACUTELY ISOLATED RAT HIPPOCAMPAL NEURONES. Michael Fasteau<sup>a</sup>, Sergey Smirnov<sup>a</sup> and Kai Kaka<sup>b</sup>. Department of Biosciences, Division of Animal Physiology, University of Helsinki, P.O.Box 17, FIN-00014 Helsinki, Finland.

We have studied the effect of extracellular H⁺ on the GABA<sub>Receptor-mediated chloride conductance in acutely isolated pyramidal neurons from the rat hippocampus under whole-cell voltage clamp in HCO₃⁻-free solutions. The conductance evoked by saturating concentrations (500-1000 μM) of GABA showed a marked sensitivity to pH variations in external pH (pH<sub>e</sub>) around 7.4. A rise in pH<sub>e</sub> between 6.4 and 8.4 decreased the conductance by about two-fold per pH unit. However, when evoked by low GABA concentrations (1-10 μM) the conductance increased by an equally marked increase upon an increase in pH<sub>e</sub>. At an intermediate concentration of GABA (around 30 μM), the GABA<sub>e</sub> conductance was not affected by external H⁺. The concentration-response relationship was that between 500-1000 μM GABA consistent with the presence of at least two functionally distinct GABA<sub>e</sub> receptors with different affinities. The effect of pH<sub>e</sub> was best described as a combination of two effects: i) a downmodulatory effect of H⁺ seen as a parabolic shift to the right in the concentration-response curve and the high affinity GABA<sub>e</sub> receptor, and ii) an upmodulatory effect of H⁺ seen as a non-competitive potentiation of both the higher and the lower affinity receptor populations. Zn²⁺ (1-50 μM) inhibited in a concentration-dependent manner the conductance induced by saturating GABA concentrations. In addition, at pH<sub>e</sub> 8.4 Zn²⁺ induced a parallel positive shift in the GABA concentration-response curve. In the presence of Zn²⁺ the GABA<sub>e</sub> conductance was upmodulated by H⁺ at both high and low agonist concentrations. The above data imply a coexistence in these cells of two functionally distinct GABA<sub>e</sub> receptor populations with different affinities to GABA and different sensitivities in H⁺ and Zn²⁺. The high sensitivity of GABA<sub>e</sub> receptors to H⁺ suggests that the efficacy of central inhibitions depends on the regulation and modulation of interstitial pH in the brain.

804.12
INHIBITORY POSTSYNAPTIC POTENTIALS IN RAT SUBCORTICAL BURSTING NEURONS. D. Martina<sup>a</sup>, H. Kawakatsu<sup>a</sup> and M. Avoli<sup>b</sup>,<sup>c</sup> Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, MONTREAL, Canada H3A 2B4.

Intracellular recordings from rat subcortical bursting neurons (BNs, n = 35) were made in an in vitro slice preparation to evaluate the inhibitory component of the response to single-shot extracellular stimuli delivered in different portions of the CA1 subfield. Stimulation of the CA1 afferents and CA1 stratum radiatum evoked a sequence of depolarizing-hyperpolarizing potentials in 17 BNs, whereas only a monophasic depolarization was observed in 18 BNs. Stimuli applied either to the CA1 stratum pyramidale or stratum lacunosum-molecularis induced in the same cells (n = 2) a depolarizing potential and a depolarizing-hyperpolarizing sequence of potentials, respectively. When varying the resting-membrane potential the stimulus-induced hyperpolarization began as a hyperpolarizing postsynaptic potential (IPSP) had a reversal potential of -81±5 mV (n = 10) and was associated with an increase in membrane conductance of 25±14 nS (n = 5). Shunt of the action potential by the depolarizing current injection occurred when hyperpolarizing IPSPs were elicited by concomitant synaptic stimulus. Bath application of the GABA<sub>e</sub> receptor antagonist BMI (10μM, n=4) reduced both the amplitude and the associated increase in membrane conductance. These findings suggest that only a subset of subcortical BNs respond with a hyperpolarizing IPSP when activated by the stimulation of the CA1 subfield. They also indicate that this hyperpolarizing IPSP is mainly due to the postsynaptic activation of GABA<sub>e</sub> receptors located on BNs. Supported by Savoy Foundation and MRC of Canada.

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804.13

A BICUCULLINE- AND SACLOFEN RESISTANT GABA CURRENT IN SUBSTANIA GELATINOSA OF THE RAT SPINAL CORD IN VITRO

G. Macdonald, M. Yoshimura, H. Baba, Y. Yaginuma and H. Higashi

Stimulation of Aβ afferent fibers evoked a GABAergic IPSP via the GABA_A receptor. In many SG neurons, however, the GABA-induced response was in part resistant to bicuculline and saclofen. To study the property of the GABA response, blind patch clamp recordings were made from SG neurons in the transverse spinal cord slices of the adult rat. Bath applied GABA (1-100 μM) produced an outward current which consisted of an initial peak and a slowly decaying plateau. The initial peak current was depressed by bicuculline (10 - 100 μM), while the plateau current was hardly affected. GABA receptor antagonists, picrotoxin (500 μM) and saclofen (500 μM) also had no significant effect on the plateau current. Picrotoxin (60 - 100 μM) depressed the GABA current and an outward current was reversed in polarity near the Cl- equilibrium potential. It appears, therefore that the plateau current has a similar property to the GABA current reported recently in visual system. The GABA_A receptor antagonist, 4-aminopyrrolidinacetic acid (CACA, 0.5 - 1 mM), in fact produced in SG neurons a response similar to the GABA response. The CACA induced current was, however, completely abolished by bicuculline (20 μM). These observations provide an idea that SG neurons may express a novel GABA receptor which has a different pharmacological property from the GABA_A receptor and that this receptor may be involved in modulation of nociceptive transmission in the spinal dorsal horn.

804.15

PENTOBARBITAL MODULATION OF GABA-A RECEPTOR KINETICS USING ULTRAFAST ACTIVATION IN EXCISED PATCHES OF MOUSE CORTICAL NEURONS

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Steady state applications of pentobarbital (PB) have previously been shown to increase GABA-A-mediated chloride current by increasing single channel open duration without changing open frequency [Macdonald et al., J. Physiol., 1989]. An ultrafast ligand exchange system (100ms exchange time) was used to examine the effects of PB on GABA binding and the GABA-A receptor opening (i.e., channel activation and relaxation). Outside-out patches from cultured fetal mouse cortex contained >10 channels and were voltage clamped at a -75mV in symmetrical chloride solutions at room temperature. GABA (10μM-10mM) alone or in the presence of PB (50μM) was applied to patches using either a series of repeated brief pauses (600us) or single step applications (50ms-2s). Chloride currents were sampled at 20-100kHz and filtered at 2-10kHz. Current onset (10-90% rise time) and decay time constants (τ) were analyzed individually or as patch ensemble averages.

Pre-application of PB was necessary to see an alteration of kinetics, suggesting that PB binds the receptor more slowly than GABA. As we previously reported for diazepam, PB did not alter channel maximal opening rates. Unlike diazepam, PB prolonged current relaxation indicating that PB increases time spent in one or more bursting states, similar to that seen for steady state single channel recordings.

NEUROPEPTIDE LOCALIZATION: CNS REGULATION

805.1

DISTRIBUTION OF THE NEUROPEPTIDE Y2 RECEPTOR mRNA IN RAT BRAIN

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V. She, A. J. Ily, R. W. Weinshank and T. A. Branchek.
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The expression cloning of the human NPY Y2 receptor cDNA and the subsequent cloning of the rat receptor (C. Gerald et al., K. E. Smith et al., Soc. Neurosci. Abstr. 1995) have made it possible to localize the mRNA encoding this NPY receptorsubtype in rat tissues. We have carried out in situ hybridization studies using [35S]-labeled oligonucleotide probes to the rat Y2 receptor mRNA to determine the distribution of Y2 mRNA in the rat brain and peripheral tissues.

Probe specificity was confirmed by testing the radiolabeled oligonucleotides in transfected cells. In rat forebrain, Y2 mRNA is most abundant in the CA1 region of the hippocampus, the pyramidal matrix of the amygdala, and in the arcuate nucleus and tuber cinereum of the hypothalusa. Hybridization signals are also observed over the olfactory tubercle, the lateral septal area, the basomedial and cortical nuclei of the amygdala, the dorsolateral and ventromedial hypothalamus, the dorsal and ventral premammillary nuclei, the perifornix cortex, and the entotor splice and paraventricular nuclei of the thalamus. Caudally, hybridization signals for the Y2 mRNA are restricted to the dorsal raphe and the pontine nucleus, and the posterior dorsal tegmental nucleus. In the spinal cord, labeling is observed over scattered large neurons in lamina 9. A population of large neurons in the dorsal root ganglia and a few granular cells in the spinal cord contained Y2 mRNA.

The present results indicate that the mRNA encoding the Y2 receptor is selectively localized in the rat brain. In some areas, co-localization of this novel receptor with NPY itself appears likely, particularly in the arcuate nucleus of the hypothalamus. The distribution of this NPY receptor subtype mRNA suggests involvement in multiple physiological roles.

805.2

ASSOCIATIONS BETWEEN NEUROPEPTIDE Y NEURAL ELEMENTS AND MICROVESSELS IN RAT AND HUMAN CEREBRAL CORTEX. D. Abouzaid* & E. Hamel.
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Neuropeptide Y (NPY) contains central intracerebral arterioles (Dacey et al., 1988). Carotid Blood Flow Method R. 2.54) and NPY-containing nerve terminals have been incidentally observed in association with intrahypothalamic microvessels (Aoki et al. 1989, J. Neurobiol. 20). To assess the morphological basis for a putative role of NPY in the regulation of microvascular function, we performed a quantitative study of the associations of NPY neural elements with microvessels in human and rat cerebral cortex, respectively. Tissue from each species was immersed in 2 perivascular. Brain sections were immunostained for NPY and analyzed for light microscopy. On 20 µm-thick sections, the overall association of NPY neurons with local intracerebral microvessels was evaluated. On semithin (2 µm) sections, the total number of NPY neuron elements, blood vessels and perivascular NPY punctate structures were counted on a light microscope. Only neuronal elements which directly touched a vessel wall were considered to be perivascular. In the 20 µm-thick sections of rat cerebral cortex, many NPY-containing nerve terminals showed associations with intraparenchymal vessels, those were more frequent between NPY processes and vessel walls, than between cell bodies and vessels. On semithin sections in the rat, 3.6% of all NPY punctate structures (n=4700) were perivascular. Also, of all vessels counted (n=470), 22.5% were contacted by least one NPY element with 6% of the vessels having 2 or more NPY processes. In map, an extremely dense network of NPY-containing neuronal elements with very few cell bodies was observed in both thick and semithin sections. Due to the inability to visualize all intracerebral vessels and to the very high density of the NPY-ir neuronal network in the human cortex, a quantitative analysis was not performed. However, many of the processes, most likely NPY terminals, were apposed to a vessel wall in semithin sections. The morphological data may indicate that cortical microvascular functions are regulated by NPY neuronal elements. Further, studies at the ultrastructural level would help define these NPY neurovascular relationships.

Supported by MRC Canada (QP & SK) & Cho-Geigy Canada Ltd. (KE)

Neuropeptide Y (NPY) and its homologues induce complex effects in the hippocampal formation that include the modulation of glutamatergic neurotransmission (Dumont et al., Brain Res. 655:57, 1994) and sigma receptor systems (Monnet et al., J. PET 243:1219, 1992; Bouchard et al., J. Neurosci. 13:5067, 1993). Among these receptors, Y1 and Y2 mediate these functional effects as both subtypes are expressed in the rat hippocampus, albeit each at a unique anatomical profile (Dumont et al., J. Neurosci. 13:5073, 1993). In order to investigate the therapeutic role of Y1 and Y2 receptors in the cellular and molecular levels, a rat embryonic primary hippocampal neuronal cell culture model was developed and shown to be uniquely enriched with Y1(123)I[Leu]3-Pro2NPYY binding sites (St-Pierre et al., Soc. Neurosci. Abstr. 1994). The aim of the present study was to establish the phenotype of Y1 receptors-bearing hippocampal neurons using a combined immunocytochemical-elsulation receptor autoradiographic method and focusing first on the possible existence of NPY-immunoreactive neurons in the culture. Dissociated hippocampal cells from embryonic day 18-19 rats were grown for 20 days on cover-slips. Emission receptor autoradiography was then performed using the selective Y1 radioligand, [125I]Leu3-Pro2NPYY (35nM), followed by NPY-immunocytochemistry (ABC method, Vector) with a highly specific NPY antibody (generously provided by G. Pelletier, Laval Univ.). Most interestingly, a significant proportion of medium- to large-size neurons were clearly NPY-immunoreactive and expressed the Y1 receptor sub-type. These results suggest the possible existence, via Y1 receptors, of an autoregulatory mechanism governing neurotransmission in the rat hippocampal formation. (Supported by the BCCRC and the FCAR.)

805.5 [125I]BIBP326, A NEWLY DEVELOPED NON-PePTIDE NEUROPEPTIDE Y Y1 RECEPTOR ANTAGONIST: RADIOISOTOPE BINDING CHARACTERISTICS AND QUANTITATIVE AUTORADIOGRAPHY. Y. Dumont* and R. Quirion, Douglas Hospital Research Centre. Dept. Psychiatry, McGill University, 6877 Ashburn Blvd., Montreal, Quebec, Canada, H3G 1R3.

Rudolf et al. (Eur. J. Pharmacol. 391: 81-90, 1999) recently reported the development of a highly selective non-peptide neuropeptide Y (NPY) Y1 receptor antagonist, devoid of activity on the Y2 and Y3 receptors. We have now carried out the characterization of BIBP326, a non-peptide NPY antagonist that shows high affinity for the Y1 receptor (IC50 of 1 nM) but not Y2 (1 µM) binding sites. Autoradiograms revealed that 100 nM BIBP326 competed for at least 75% to 90% of 30 nM [125I]Leu3-Pro2NPYY, against binding sites in the superficial laminae of the lateral septum, nucleus tractus solitarius (NTS) and area postrema are rather resistant (<30%) to BIBP326. The direct use of [125I]BIBP326 (5 nM) as radioligand confirmed and extended these findings with high specific labeling concentrated in cortical areas and in the thalamus for example, but not in the NTS and the area postrema. Taken together, these results may suggest the possible existence of Y2 receptor subtypes, one being highly sensitive to BIBP326. Supported by an NSERC Industry/University Grant jointly with Thomas Goebel-Bio-Mega.


A radioligand binding study has shown that sciatric nerve sectioning, [125I]BIBP326 binding decreased, while [3H]NPY binding increased in the superficial laminae of the ipsilateral dorsal horn (Kar and Quirion, Brain Res. 574:333, 1992; Zhang et al., Eur. J. Neurosci. 7:567, 1995). In order to determine the neuropeptide Y (NPY) receptor subtypes involved, the respective distribution of the Y1 and Y2 receptors was investigated in the rat and human spinal cord using recently developed selective and sensitive assays (Dumont et al. J. Pharmacol. 267:272, 1993). Another series of experiments were carried out to determine whether Y1 and Y2 receptor binding sites are differentially altered following sciatric nerve ligation and in ALS spinal cord. Autoradiographic studies revealed that high levels of both Y1(123)I[Leu3-Pro2NPYY] and Y2(3H)NPY binding sites are present in the superficial laminae of the dorsal horn and ipsilateral spinal cord. Deeper laminae and the ventral horn are more heavily labelled with Y1 ligand. Following unilateral sciatric nerve section (14 days), specific Y2 binding decreased while Y1 labelling increased in the superficial laminae of the ipsilateral dorsal horn. In contrast to the rat spinal cord, Y1(123)I[Leu3-Pro2NPYY] and Y2(3H)NPY binding sites are similarly rather distributed in the normal human spinal cord with labelling particularly concentrated in lamina I and II, and the ventral horn expressing low but still significant levels of binding sites. No significant alterations were noted in the distributional profile and/or density of Y1 or Y2 receptor subtypes in the ALS cord. Taken together, these results suggest that both the Y1 and Y2 receptor subtypes are expressed in the rat and human spinal cord, and are differentially regulated following sciatric nerve lancing. Supported by the NSERC and an Industry-University grant.

805.7 DISTRIBUTION OF [H]NEUROTENSIN RECEPTORS IN HUMAN BRAIN. Susan R. Bachus, Carol A. Tammings and Robert A. Lajtha, Maryland Psychiatric Research Center, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228.

The distribution of receptors for neurotensin in human brain are distributed primarily in so-called limbic areas, with a distribution that appears to be distinct from that of dopamine receptors. We have examined dopamine receptor subtype distributions within human brain, and would like to compare these distributions with that of neurotensin receptors in brain tissue from normal controls. We present here autoradiographic data using [3H]neurotensin ([H]-NT) to locate NT receptors in normal human brain. Two cortical homi-blocks were cut at the level of the base of the lateral at the level of the hippocampus from frozen human brain tissue from three individuals with no known psychiatric or neurologic illnesses. Each homi-block was divided into 3 smaller blocks, and these were sectioned at 20μm on a cryostat, thaw mounted, incubated in [H]-NT, and developed on tritium-sensitive autoradiographic emulsions were exposed to EMI high-contrast Emulsion film (ARC). Binding across blocks and cases. Areas of intense binding include anterior cingulate cortex (ACC), insular cortex, and entorhinal cortex (ERC), with a greater density in the superior frontal, prefrontal cortex (PFC), bindings and ACC and PFC superficial cortical layers was about twice that in deep layers. Discrete areas of intense binding within the superficial layers of ERC were consistent with localization to the layer II cellular islands. These data are consistent with previous reports of distribution of [3H]-NT receptors in human brain tissue.

805.8 NEUROTENSIN RECEPTOR RNA LOCALIZATION IN HUMAN MIDBRAIN. BASAL GANGLIA. CLINOCATE CYRRIID AND HIPPOCAMPAL FORMATION BY IN SITU HYBRIDIZATION. S.P. Wolf*, S. Bachus, B. L. Korkeakallio, N. M. Ownby, and C.B. Nemeroff. J.E. Kleiman1, T.M. Brady1. NHM Neuroscience Center at St. Elizabeths, Washington, DC 20032 and Dept. of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322.

Neurotensin (NT) is a putative CNS neurotransmitter which may be implicated in certain neuropsychiatric diseases. High concentrations of NT receptors are present in specific brain regions, including the substantia nigra (SN), ventral tegmental area (VTA), entorhinal cortex and cingulate cortices, with moderate concentrations in the basal ganglia. Recently, human midbrain and striatal NT mRNA expression has been found to be up-regulated in in situ hybridization (Yamada et al., Neuroscience 64:405-417, 1995). We have corroborated these findings and further examined select limbic regions in which NT receptors have previously been shown to be up-regulated. Using a highly sensitive antisense riboprobe, we examined several rostral-caudal levels of 8 normal human midbrain. High levels of NT receptor mRNA signal were detected in the substantia nigra pars compacata and in the paraganstant substantia nigra in SN. The mRNA signal was detected in other subnuclei of the VTA and other mesencephalic structures. No significant signal was observed in the lateral geniculate, inferior colliculus or pontine nuclei. The NT signal was seen in the dentate gyrus, with light labeling of layer II of the entorhinal cortex. Preganglionic cingulate cortex revealed only faint signal in a homogenous pattern. The association of high NT receptor mRNA signal and dense NT receptor concentrations in some regions, such as SN, is consistent with a perikaryal localization of NT binding sites. In contrast, the widespread neuronal density in the substantia, cingulate and entorhinal cortices, with faint, or absent, mRNA signal suggests that these regions may represent terminal NT binding sites.
805.11

The number of striatal neurons with COX-like immunoreactivity is strikingly increased in substantia nigra in rats. Immunohistochemical staining with antibodies against COX-1 and COX-2 was performed on formaldehyde-fixed, paraffin-embedded sections of the rat brain. We found that the number of COX-immunoreactive neurons was significantly increased in the substantia nigra compared to control animals. In addition, we observed that the number of COX-immunoreactive neurons was also increased in the substantia nigra of rats with Parkinson's disease. This increase in COX-immunoreactive neurons may be related to the pathological changes associated with this disease.

805.13

IMMUNOLocalIZATION AND QUANTITATION OF FIBROBLAST GROWTH FACTOR RECEPTOR-1 IN THE HUMAN PERIPHERAL NEUROMA Q Ma R.W. Baumgard, S. Zhao, I. Pedrosa, H. Tran, D. Nguyen, D.G. Kline, Dept. of Ophthalmology, LSU Eye Center, New Orleans, LA 70112

Immunocytochemical localization of fibroblast growth factor receptor-1 (FGFR-1) was studied in human neoplasms using techniques of the art. The cultured cells consisted of large numbers of fibroblasts and a few Schwann cells identified by DAPI staining. Some of the results showed that the fibroblasts were positively stained using an immunohistochemical procedure with a monoclonal antibody to recognize FGFR-1. Further, the subcellular location of FGFR-1 in the cells was investigated by electron microscopy. FGFR-1 was found to be present in the cytoplasm of the fibroblasts as discrete aggregates. The receptor density was calculated to be about 5600 per cell. The results will be useful for determining the function of FGFR receptors in human neoplasms. Supported in part by DAMD17-93-V-3013.

805.14


Antisera generated against Xiphophorus neurotrophin-6 was utilized to study the distribution of nerve growth factor (NGF) in the brain and pituitary gland of Xiphophorus maculatus, the platyfish, at different stages of sexual development. In both immature and mature animals, NGF was found in an intensely stained segment of the pituitary in the anterior lateral nucleus lateralis terminals (LNT). Processes of these cells extended ventromedially toward and through the infundibulum and into the neurohypophysis of the pituitary gland. In the pituitary, immunoreactivity was seen in the pars intermedia and in the gonadotropic zone of the caudal pars distalis. Immunoreactivity was also noted in the hindbrain of male animals. In neonatal animals, ir-granules were also noted around cells of the anterior olfactory lobe. These data suggest that NGF is involved in pituitary gland physiology, from early embryogenesis to sexual life. (Supported by NASA (NAGW-1704) and BARD (IS-2149-92)).
A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the rat brain. A.M. Gonzales*, M. Berry*, L.A. Wainman. A.Scripps Research Institute and the Department of Cell Biology, La Jolla, CA; UMDSD (Guy's Campus) London, UK; Dept. of Clinical Chemistry, Umea, Sweden.

We examined the distribution of basic fibroblast growth factor (FGF-2) and its receptor (FGFR1). The results confirm the widespread distribution of both, and different regions express distinct patterns of protein: neuronal and non-neuronal. The distribution of FGFR1 mRNA is consistent with the protein expression, indicating FGFR1 plays a role in neuronal growth factor signaling.

**Peptides: Physiological Effects IV**

### 806.1

**INCREASED VASOPRESSOR ACTIONS OF NEUROPEPTIDE Y-(13-36) IN SPONTANEOUSLY HYPERTENSIVE VERSUS NORMOTENSIVE RATS MAY BE DUE TO INCREASED V2 RECEPTOR BINDING IN THE NUCLEUS TRACTUS SOLITARIUS.** F.B. Hollgård, J.A. Apulenti, I.A. Ardourel, and K. Ueda. Dept. of Neurosurgery, Karolinska Institute, Stockholm, Sweden and Dept. of Physiology, Univ. of Málaga, Málaga, Spain.

The C-terminal NPY fragment (3-36) (NPY(3-36)), a NPY V2 receptor agonist, elicits natriuretic and antihypertensive effects. We have studied the cardiovascular responses of NPY-(3-36) and the distribution of NPY receptor subtypes within the nucleus tractus solitarius (NTS) in spontaneously hypertensive rats (SHR). NPY(3-36) was injected intracisternally at fixed infusion rates (7.5 to 3000 pmol) in awake, unrestrained rats to evaluate the cardiovascular effects. NPY receptor subtypes were studied by autoradiography using 125I-substituted Y2 antagonist (I25IYY) as a radiolabeled agent and by measuring the Y1 and Y2 receptor subtypes with unlabelled [3H] and [3H]NPY and NPY-(3-36) respectively. In both male SHR and age-matched male normotensive Wistar Kyoto rats (WKY) NPY-(3-36) injections elicited vasopressor effects. In WKY this effect was dose-dependent and became significant at doses from 75 pmol, whereas in the SHR the vasopressor effect had a longer duration than in the WKY and became significant at lower doses (25 pmol) but associated with the development of an early ceiling effect. The heart rate was unaltered in both groups of rats. Total specific [3H]Y2 binding in the NTS was 25% higher in SHR than in WKY rats. By masking the Y1 and Y2 receptor subtypes respectively it could be shown that the difference was due to V2. In Y2 receptor binding within the NTS. The present results give evidence for an increased potency but not an increased efficacy of NPY-(3-36) in inducing a pressor response in SHR compared to WKY rats and suggests that the V2 receptor system is upregulated in SHR compared to WKY. These enhanced vasopressor effects may partly be explained on the basis of an increased density of Y2 receptor (vasopressor effect) versus Y1 receptor subtype (vasodilatory effect) leading to different sensitivity reactions in the SHR. The peak activity of NPY-(3-36) in SHR may not be increased as the already high blood pressure levels in these rats.

### 806.2


Arcuate and hilar NPY mRNA expression has been shown to be regulated by adrenocorticotropin (ACTH) and a week infusion of adrenal steroids (Wantanabe et al., Mol Brain Res (1995) 28:135-140). ACTH caused an increase in NPY mRNA whereas NPY mRNA decreased in the arcuate. We investigated whether acute stress would also exert a differential effect on NPY mRNA expression in the hippocampal hilus and arcuate nucleus. Male rats were acutely stressed by placing them in wire mesh chambers for 1 hour. Rats were decapitated at 0 (no stress), 1, 6 and 24 hours after restraint. In situ hybridization was used to determine NPY mRNA levels. Film analysis of the autoradiograms revealed that NPY mRNA increased in the arcuate 24 hrs after one exposure to restraint stress, F(3,8)=3.247, p<0.05. NPY message was not elevated 1 and 6 hours after restraint compared to non-stressed rats (10 rats/group). NPY mRNA increased in the dentate hilus 6 and 24 hrs after restraint compared to the non-stressed and 1 hr groups, F(3,19)=3.747, p<0.05 (5-6 rats/group). Preliminary data from grain counts revealed that mRNA CORT levels indicated that serum CORT was elevated immediately following the stress (1 hr rats = 41.6 g/ml) and that these levels declined by 6 hours. These data indicate that CORT levels are sufficient to elevate NPY mRNA levels in both the arcuate nucleus and hippocampal hilus. Furthermore, acute stress did not differentially alter NPY mRNA expression between the arcuate and hilus. Supported by grants MH10804 to CC and MH14125 to BM.
086.3  

Although the identity of the endogenous ligand for sigma receptors is controversial, various neuropeptides and steroids have been shown to compete for binding to these receptors. The sigma receptor ligand candidates is neuropeptide Y (NPY) which mimics electrophysiological preparations of some sigma ligands. We have previously shown that specific sigma agonists such as (+)-pentazocine and BDZ77 inhibit stimulated [3H]DA release in various brain regions. Using a superfusion system, we further investigated the effects of NPY on stimulated [3H]DA release to the effects of these ligands. In contrast to (+)-pentazocine and BDZ77-mediated inhibition of release, NPY enhanced release at nM concentrations. Several sigma antagonists reversed the enhancement. Effects of NPY receptor antagonists are currently being tested. (Supported by a NIDA grant to LHW.)

086.4  

Three angiotensin receptor subtypes have now been identified and characterized within the brain: AT1-angiotensin II (AngII) binding sites that are coupled to the AT1 subtype, AP, appear to be responsible for mediating the classic physiologic and behavioral responses associated with the brain RA, namely pressor and drinking responses, vasopressin release, salt appetite, and sexual behavior accompanied by cyclic production of reproductive hormones. It has been assumed that angiotensin II (AngII) activates the AT1 receptor subtype, although there is evidence that angiotensin III (AngIII) may also act at this site (reviewed by Chiu et al., 1993). The present investigation utilized three metabolically stable sigma agonists in an attempt to determine the form of angiotensin that activates the centrally mediated pressor response. Each of these analogues was modified at the N-terminus with a hydroxylsulfurane group (CHOHCH2NH) residue bond. This bond primarily differs from the peptide bond in that it permits free rotation of the backbone bonds, it is 1.67 anagrams longer, and it significantly increases the half-life of each molecule. Intracerebroventricular injection of each analogue (100 pmol/min) for 10 min in alert rats indicate maximum mean (SEM) systemic blood pressure elevations of 20±5.7, 72±6.7, and 17±0.9 mm Hg for AngII, AngIII, and IV pseudopeptide analogues, respectively. Comparable values for native AngI, AngII, and IV were 24±2.2, 21±3.1, and 15±1.1 mm Hg. These results suggest that native AngII and AngIII are equipotent at the AT1 receptor subtype, while AngIV is less potent. The advantage offered by AngII over AngII may be that it activates this receptor as AngII, and is then converted to AngIII that also activates the receptor, followed by conversion to AngIV which is briefly activated. AngII does not offer this "multiple-ligand" effect.

086.5  
THE EFFECTS OF ESTROGEN ON ANGIOTENSIN II STIMULATED PROLACTIN SECRETION IN VITRO. C.E. Bryant, K. Zochdorff and P. Cellianni*. Dep. of Pharmacology, Center for Neuroscience, Miami University, Oxford, Ohio 45056.

The role of Angiotensin II (AngII) in the physiological regulation of Prolactin (PRL) release is unknown. The purpose of these studies was to determine the effect of estrogen on the all- induced PRL secretory response from dispersed anterior pituitary cells. Female rats were ovariectomized (ovx) at 4-6 weeks of age and divided into 2 groups. One group of ovx females received estrogen replacement pellets (ovx+E), while a second group received placebo (ovx). A third group of female rats was left intact. Two months later, the stimulatory effects of all on PRL release were determined in vitro. Administration of AngII significantly stimulated PRL release in cells obtained from intact female donors. All did not stimulate PRL secretion from cells obtained from ovx+E treated females and was only weakly stimulatory to cells obtained from ovx rats. These results indicate that estrogen alone is not responsible for the sensitivity to all stimulation observed in cells from intact female rats. Also, All did not stimulate PRL secretion from cells obtained from male rat donors. Scatchard analysis of 125I-AVD binding revealed no difference between Kd and Bmax values in male and female anterior pituitaries. Thus, it appears that anterior pituitary cells from female rats are sensitive to all stimulation, but that estrogen is not solely responsible for this sensitivity. This response to all stimulation does not appear to be a receptor mediated phenomenon since there was no difference in receptor binding characteristics (Kd and Bmax values) between pituitaries obtained from male or female rats. (Supported by NIH grant DK 48023 to PC.)

086.6  

Vasopressin is intimately involved in the hypothalmo-pituitary adrenal axis. V1a vasopressin receptors (V1aR), the primary subtype expressed in the brain, and their mRNAs, are expressed in V1aR-1 macrophages. Both the receptor and its mRNA are enhanced by dexamethasone (DEX) treatment. To examine the molecular basis of this effect, we have isolated a genomic clone encoding the V1aR. Approximately 3.5 kb have been sequenced revealing the presence of 3 putative glucocorticoid response elements (GREs) in the 5' flanking region of the gene. Gel mobility shift assays using these putative GREs and nuclear extract from DEX treated WRK-1 cells indicate that 2 of the 3 putative GREs are active in protein binding and might mediate transcriptional effects of glucocorticoids. The septum of the rat brain expresses a vasopressin V1aR which has been implicated in various rodent behaviors. In order to examine in vivo effects of adrenal steroids on septal V1aR, we bilaterally administered (ADX) rats either saline or DEX. Hormone replaced them with either DEX in different concentrations, or with aldosterone. The effects were evaluated in the septum of these animals using a radiolabeled specific V1aR antagonist 125I-Tyr3-AVP. ADX significantly decreases (p<0.05) V1aR binding site density below those of the sham ADX controls. Additionally, DEX, but not aldosterone, was able to restore V1aR binding in ADX animals to levels comparable to those of sham controls. Studies are currently in progress to determine the effects of these steroids on V1aR mRNAs in the septum. (Supported by NS23113, the VA and Pharmacological Sciences Training Grant 5T42HD049).

086.7  

The neuropeptide arginine vasopressin (AVP) is thought to play an important role in HPA axis regulation at the level of the paraventricular nucleus (PVN). The release of AVP within the PVN in response to physical and emotional stressors was assessed using microdialysis in adult Wistar rats. Compared to pre-stress values (0.1-1.0 pg/30/min dialysate), swimming in the Morris water maze increased AVP release (380%, p<0.01, n=8). Intra-PVN release of the neuropeptide peaked also during social defeat (226%, p<0.01, n=11), but was just slightly increased during exposure to "mildly" stressful (p<0.16%) exposure to a juvenile (1600% n.s.; novel environment: 137%, n.s., n=8 each). These data provide evidence that both physical and emotional stressors are potent to intracranial AVP release and confirm the hypothesis of an involvement of the intracranially released neuropeptide in coping with intense stress. Since plasma levels of AVP are known to rise by intracerebroventricular injection of the HPA axis in response to all stressors used (Morris water maze: 574%, p<0.01, n=6; social defeat: 510%, p<0.01, n=6; juvenile exposure: 258%, p<0.01, n=10; novel environment: 424%, p<0.01, n=6) further studies have to investigate to which extent AVP release within the PVN contributes to the regulation of the HPA axis activity. Supported by VW.
806.9

OXYTOCIN ANTISENSE REDUCES SALT INTAKE IN THE BARORECEPTOR DERENERVATED RAT. M. Morris*, P. Li, C. Barrett, M.F. Callahan, Dept. of Physiology and Pharmacology and The Hypertension Center, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157

Interruption of baroreceptor input to the brain produces a state of salt deficiency. In view of the role of oxytocin (OT) in the control of salt intake and excretion, experiments were performed to evaluate the effect of central injection of OT antisense oligomers on reduced salt intake shown in sinoaortic denervated rats (SDA). Paraventricular injection of OT antisense (AS) decreased intake of 2% NaCl in the SAD, but not in the sham operated (SG) controls. The SAD rats consumed 22±4 ml after mixed base control injection as compared to 8±4 ml after the OT AS injection (p < 0.05). There was no difference in the response of the SG controls. The SAD animals demonstrated an increase in the plasma OT response to 24 hours salt loading (3.2±0.7 to 6.9±0.8 pg/ml as compared to 2.8±0.9 to 4.4±0.9 pg/ml, SAD vs SG). The increased endocrine response occurred even though salt intake was lower in the SAD animals. There were no group differences in plasma electrolytes or posterior pituitary OT content. Results demonstrate a specific effect of OT antisense in the denervated animal, suggesting that central OT stimulates salt intake in this model.

(Supported by NHLBI Grant #HL-43178).

806.11

ASTRESSIN, A POTENT CYCLIC CRF ANTAGONIST


Predictive methods, physiochemical measurements (NMR, CD) and structure activity relationships studies suggest that corticotropin-releasing factor (CRF) and its family members (urotensins and sauavagine) assume an α-helical conformation when interacting with the CRF receptors. We have scavenged the rCRF sequence with several constraining modalities such as an (i+4)3 and (i+4)4 bridge consisting of the D or L-Glu-Xaa-Xaa-(Xaa)-D or L-lys scaffold. From this series we have identified a unique substitution that resulted in an antagonist, Astressin (a 30-peptide) that is >100 times more potent than the α-helical CRF-41, 42 times more potent than β-Phe6-11 Nal13,18-Bol-11,12-CRF-41, our present standard, and 400 times more potent than the corresponding linear analog in an in vitro pituitary cell culture assay. As expected, Astressin had very low affinity for the CRF binding protein and very high binding affinity in a specific receptor assay. In vivo, Astressin is significantly more potent and longer acting that any previously tested antagonist at inhibiting basal or stimulated ACTH secretion. Indeed, Astressin blocked stress-induced ACTH release at less than 0.3 mg/kg, iv in the rat. In an agonist (a 38-peptide), this structural modification resulted in a 2-fold increase in potency as compared to the parent linear analog. The differences in relative potency in the agonist (2-fold loss) and antagonist (400-fold loss) series between the linear and cyclic forms suggest that the bridging reconstitutes a structural motif in the agonist equivalent to that brought about by an unaltered N-terminus octapeptide in the agonist.

CATECHOLAMINE RECEPTORS: GENETICS

807.1

THE STATUS OF THE Dopamine D2 RECEPTOR (DrD2) LOCUS AS A SUSCEPTIBILITY FACTOR IN AUTISM: FAMILY ASSOCIATION STUDIES.

S.D. Flanagan*, E. Cook, C. Courtinche, A. Lincoln, C. Lord, B. Leventhal, R. Cronin* and R. Courtinche, 1Beckman Res. Inst. of the City of Hope, Duarte, CA;2University of Chicago Hospitals, Chicago, IL; and 3Children's Hospital, San Diego, CA

Case-control studies support the hypothesis that a genetic variant at the D2DR2 locus contributes significantly to risk for autism. The hypothesized D2DR2 susceptibility factor is neither necessary nor sufficient to cause autism and probably interacts with other factors, genetic or non-genetic. Linkage disequilibrium with the DRD2 Taq1A allele is the subject of considerable controversy concerning its role as a risk factor in severe alcoholism. Much of the controversy revolves around limitations inherent in any genetic association study utilizing the case-control design. In severe alcoholism, there is the additional problem that variations in subject recruitment for alcoholics possessing different risk factors, explaining the wide divergence in various reports. By contrast, autism as diagnosed by well established ADOS and ADOS criteria, is unlikely to be affected by vagaries of subject recruitment; furthermore, the parents of autistic subjects are readily recruited providing the opportunity for the calculation of haplotype relative risk in the powerful family association design. We recruited 31 sets of autistic patients and their parents (Courtinche lab) and found a trend for elevated transmission of the DRD2 Taq1A allele to probands (z=3.05, p=0.001). Data for the DRD2 Taq1B allele, which lies in strong linkage disequilibrium with the Taq1A allele, were as follows: 12 B1 and 50 B2 parental alleles were transmitted to probands; 3B1 and 59B2 alleles were not transmitted (Fisher exact, one-tailed, p=0.001). We have now recruited a separate, replication sample of 27 sets of probands and parents (Cook lab) to test our hypothesis that the association of autism with DRD2 Taq1B allele exists. A portion of this work was supported by the Wacker Foundation.

807.2


The D2 dopamine receptor specific binding of [3H]-SCH 23388 was determined in discrete brain regions of both male and female C57BL/6 (B6), DBA/2 (D2), B6D2F1, F2 cross and F3 cross mice. Data were obtained for the substantia nigra zona reticulata (SNr), the core and shell of the nucleus accumbens (NAcc) and both the lateral and dorsal medial septums and the caudate-pontens (CP). N = 12/sex for the parental strains and the F1 crosses and 30/sex for the F2 crosses. Variance in receptor binding for the F2 crosses is derived from both heritable and non-heritable causes. The non-heritable or environmental component (VE) was estimated from the variances seen in the parental strains and the F1 crosses. For the F2 males, significant broad sense heritability was found in the NAc core (0.75) and the NAc shell (0.60). For the F2 females, significant heritability was detected only in the D2 strain, a difference (60%) which was most marked for the males in the NAc shell. The most marked gender effect was seen in the SNr; receptor binding for the B6 females was > 100% higher than that of the B6 males. The F2 cross was genotyped for D2 and D3, a microsatellite which is polymorphic between the B6 and D2 strain and which is closely linked to Drd1. No association was detected between receptor binding and genotype.

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The receptor specific binding of [3H]epiberline was determined in discrete brain regions of the male and female C57BL/6J (M) and DBA/2J (D2), B6V2D5 F1 and C3H/HeJ mice. Epiberline binds with high affinity to D2 and D3 but not to D1 dopamine receptors. Data were obtained for the substantia nigra zona compacta (SNc), the ventral tegmental area (VTA), the core and shell of the nucleus accumbens (NAc), and the lateral and dorsoventral aspects of the caudate-putamen (CPUs). The binding of [3H]hydroxy-4DPA was used to determine that D3 receptor binding is < 5% of the total dopamine binding in all brain regions. In general, there was no difference in the heritability of receptor binding between F1 males and females. Collapsing the data across gender, the broad sense heritability was SNC (0.56), VTA (0.50), NAc core (0.52), NAc shell (0.62), d1CPU (0.56) and ICPU (0.60). In the NAc core and shell, binding was modestly higher (10-25%) in the B6 as compared to the D2 strain. Further, compared to the B6 males, the B6 females showed a significantly higher receptor binding in the core (+25%). In both the SNC and VTA, receptor binding was markedly higher (> 50%) higher in the D2 strain; there were no gender effects in these regions. The F1 cross (N=100) was genotyped for D9M6G1 and D12M9G1, microsatellites which are polymorphic between the B6 and D2 strains and which are closely linked to Dnd2. The results show that for both the striatum and midbrain, the D2 allele is associated with higher receptor density.


The broad sense heritability of cholinergic cell density, as high as 78% in some areas of the mouse caudate-putamen (CPU), was determined from the analysis of C57BL/6J (B6) and DBA/2J (D2) inbred mouse strains and B6xD2 F1 and F2 hybrid crosses. In order to identify genes associated with this phenotype, quantitative trait locus (QTL) analysis was performed using 25 strains of the BXD/Fy series. Confirmation of the putative QTL was accomplished using 50 B6xD2 F1 hybrids phenotyped for cholinergic cell number. Two possible candidate QTL were revealed by this analysis. D5M6G21 on chromosome 5, near Dnd2, the D2 dopamine receptor gene, and D12M9G7 on chromosome 12, near c-fos, Dnd2 is associated with neurotensin-induced caudate and dopamine receptor density. c-Fos is induced by magnetic agonists and antagonists as well as dopamine antagonists such as haloperidol. Further, as reported elsewhere at this meeting (Patel et al.), in comparison to the B6 strain, the haloperidol sensitive D2 strain shows a more marked Fos response in most regions of the striatum. Overall, the QTL associated with D5M6G21 and D12M9G7 are considered good candidates a) for additional confirmation in other F1 crosses, b) for the formation of congeneric lines and c) for eventual positional cloning of the relevant gene(s).

807.6 GENETICS AND THE HALOPERIDOL-INDUCED INCREASE OF FOS IN THE MOUSE STRIATUM. S. Patel, B. Hitzmann, and R. Hitzmann*. Departments of Neurobiology and Pharmacology, Neurology and Pharmacology, SUNY at Stony Brook, NY 11794-8101.

Haloperidol and related antipsychotic drugs (those which induce extrapyramidal symptoms [EPS]) increase striatal Fos expression. The Fos response in the caudate-putamen (CPus) is blocked by anticholinergic drugs and markedly reduced after the administration of atypical antipsychotic drugs such as clozapine. These data suggest that the Fos response is predictive of EPS. An alternative method to test this hypothesis is to compare the Fos response among inbred strains of mice which are differentially sensitive to haloperidol-induced caudate (catechol is the mouse equivalent of EPS). The EDA, for haloperidol-induced caudate in the DBA/2 (D2) strain is 0.4 mg/kg and in the C57BL/6 (B6) strain is 4.0 mg/kg. Male D2 and B6 mice were administered 0.3 and 1.0 mg/kg of haloperidol, sacrificed 1 hour later and Fos expression was measured in the rostral striatum using standard immunocytochemical methods. In the lateral CPus, Fos expression in response to both doses was on average >100% higher in the D2 strain. The pattern was the same in the core of the nucleus accumbens but the magnitude of the difference was less (ca. 50%). In the shell of the NAc, the difference between the D2 and B6 strains was the same as in the core at 0.3 mg/kg but began to reverse at 1.0 mg/kg. In the dorsoventral CPus and depending on the rostral/caudal level sampled, there was either no difference between strains or the B6 strain showed a greater Fos response. Thus, only in some brain regions does the Fos response parallel the behavioral differences in strain sensitivity.

808.1 FUNCTIONAL AND BINDING STUDIES OF PHOSPHORYLATION AND GLYCOSYLATION SITE MUTANTS OF 5-HT7 RECEPTORS. E. Batt, M.A. Segalowicz, T. Green, P. Rice* and S.C.R. Lumitarski, Division of Neurobiology, LMB and Department of Zoology, University of Cambridge, Cambridge, UK.

The 5-HT7 receptor, encoded by the gene of ligand-gated ion channels (Narita et al., 1991), possess a number of potential posttranslational modifications. We have used site-directed mutagenesis to test these sites for their function in homerotic 5-HT7 receptors. In particular, we have explored the function of phosphorylation in generating differences observed in the two splice variants of the 5-HT7 receptor, which differ by 6 amino acids and a potential phosphorylation site. Full length 5-HT7AR (short) DNA was obtained from NIE-115 mINa cells using PCR. Coding sequence was the following six amino acids in the 5-HT7AR (short) subunit was inserted using on site-directed mutagenesis, as were mutations to remove potential phosphorylation and glycosylation sites. Sequences were inserted into the eukaryotic expression vector pRC/CMV and transferred into HEK 293 cells using calcium phosphate precipitation. Cell lines were characterised using radioligand binding and whole cell patch clamp electrophysiology. Radioligand binding studies with [3H]ligand showed that relative potencies of a selection of 5-HT7 receptor selective ligands were similar (GRK50; >100-2000 >5-HT7 >5-HT3 >5-HT1B/1D) in long, short and functional mutant receptors. However patch clamp studies showed differences in agonist affinity and efficacy (long > short); binding studies also distinguished between the splice variants: Kd = 0.4 ± 0.03 and 0.24 ± 0.02 (n=4) for long and short forms respectively. Phosphorylation was not responsible for this observed change in affinity. The results from the glycosylation study show that only one of the potential glycosylation sites (N919) is crucial for ligand binding function. Marini, A.V., Peterson, A.S.B. Blake et al. (1991) Science 254, 432-437.

808.2 IDENTIFICATION OF AMINO ACID RESIDUES INVOLVED IN AGONIST/ANTAGONIST LIGAND RECOGNITION BY THE 5-HT7 RECEPTOR. J.A. Steel, F.G. Bossaert, L. Stewart, M. Davies* & L.L. Martin. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. 1F Hoffmann-La Roche AG, Department PRPN, P.O. Box CH-4002, Basel, Switzerland.

The 5-HT7 receptor is a member of the ligand-gated ion channel family. To investigate the importance of certain amino acids of the agonist/antagonist ligand recognition, we have performed site-directed mutagenesis on the 5-HT7 AR cDNA isolated from NG108-15 cells. 5-HT7 receptor mutants were transiently expressed in HEK 293 cells and characterized using whole-cell patch clamp electrophysiology and radioligand binding.

The E106D mutation caused a 10-fold reduction in the KD of [3H]CGRP5630, while there was a 100-fold reduction in the KD for 5-HT in competition with this ligand, compared to wild-type. There was no change in the EC50 value for the agonist CGRP with electrophysiology. However, the Ki and EC50 values for the E106N mutation were decreased 40-fold and 10-fold, respectively, when compared to wild type. E106 appears to be particularly important in agonist recognition for the high affinity desensitized conformation of the receptor which is measured by binding, but less so for the low affinity resting conformation, which is measured by electrophysiology and is integral to channel activation.

Supported by Glaxo Canada and MRC Canada.
808.3 

SOLUBILISATION OF THE 5-HYDROXYTRYPTAMINE3 RECEPTOR RECOGNITION SITE EXPRESSED IN PIG CEREBRAL CORTEX 
S. Fletcher* & NM Barnes. Department of Pharmacology, The Medical School, University of Birmingham, Birmingham B15 2TT UK. 

5-Hydroxytryptamine (5-HT3) receptor recognition sites were solubilised from homogenates of pig cerebral cortex (1 g original weight/ml, in 25 mM Tris-HCl. 0.25 M sucrose, 1 mM CaCl2 of an equal volume of buffer (25 mM Tris, 2 mM EDTA, 100 µM PMSE, 10 µg/ml bacitracin, 10 mM sodium azide, 10 µM soybean trypsin inhibitor, pH 7.45) containing 0-4% Triton X-100. Maximum yield (356.4±3.2 nM per mg of homogenate preparation; 8.92±0.8, 7.33±0.21 and 6.4±0.12 (mean ± SEM, n=3) respectively, compared to values of 7.7±0.16, 7.4±0.05 and 6.28±0.02 (mean ± SEM, n=6) obtained using Triton X-100 at a final concentration of 0.4%.

Preliminary studies suggest that the pharmacological profile of solubilised 5-HT3 receptor sites is similar to that obtained for the receptor sites in the crude homogenate. For example, the agonists O-Me-5-HT and 5-HT, the antagonist zacopride, and the agonist 5-HT3, compete for [3H](S)-zacopride binding to solubilised receptor preparations giving similar pKs values to those obtained in crude homogenate preparations: 8.92±0.8, 7.33±0.21 and 6.4±0.12 (mean ± SEM, n=3) respectively. These results were not significantly different to the values of 7.7±0.16, 7.4±0.05 and 6.28±0.02 (mean ± SEM, n=6) obtained using Triton X-100 at a final concentration of 0.4%.

We conclude that the 5-HT3 receptor recognition site in pig brain has been successfully solubilised, which is an essential prerequisite for purification of the receptor.

S. Fletcher is recipient of an AJ Clark Studentship from the British Pharmacological Society.

808.5 

5-HT3 RECEPTOR AGONISTS REDUCE GLUTAMATE NEUROTRANSMISSION IN THE HIPPOCAMPUS OF THE RAT. B. Robinson*, E. MacIntyre, & S. Y. Wat.

We have previously shown that the 5-HT3, receptor agonist 2-methyl-5-HT reduces both EPSPS and IPSPs of CA1 pyramidal cells evoked by stimulation of the Schaffer collateral. The action is receptor specific and concentration dependent.

However, others have suggested that activation of 5-HT3 receptors in the hippocampus can increase GABA release at interneurons terminals. In the present study, we further examine the role of 5-HT3 receptors in this area using the more selective and potent 5-HT3 receptor agonist SR 57227A (SR), applied either iontophoretically or via bath. Glutamate and local neurotransmitter were used as markers of synaptic function in the Schaffer collateral and local collateral stimulation of glutamate. We used whole cell current- and voltage-clamp recordings from CA1 pyramidal neurons. Glutamate was pulsed at a current adjusted to produce sub-depolarizations of 1-1.5 mV. Similar to the action of 2-methyl-5-HT, SR consistently reduced PSIs elicited by Schaffer collateral stimulation without changing the membrane properties of the cells. Further, local intrastryosome of SR reduced glutamate-induced depolarisations by about 50% of control values, while not altering the kinetics of glutamate uptake or receptor activation or the input resistance of the cell. These actions of SR were blocked by the selective 5-HT3 receptor antagonists BRL3494 and tropisetron but not the 5-HT3 receptor antagonist (+)-WAY105535. The 5-HT3 receptor agonist 8-OH-DPAT also inhibited glutamate-depolarisations, which was blocked by (+)-WAY105135. In several cells, SR produced a slow hyperpolarization of the cell's resting potential that did not desensitise. It appears from these experiments that 5-HT3 acting at 5-HT3 receptors, will modulate glutamatergic currents in the CA1 area of the hippocampus in a manner not directly involving the opening or closing of membrane ion channels. (Supported by USPHS grants MH44440 and DA07139; K.B. was supported by NSRA fellowship F31-DA55523)

808.7 

ABILITY OF TRICHLOROETHANOL TO MODIFY 5-HYDROXYTRYPTAMINE3 RECEPTOR MEDIATED DEPOLARISATION OF THE ISOLATED RAT VAGUS NERVE. KR Bentley*, KD Johnston, RL Stevens and NM Barnes, Dept of Pharmacology, Medical School, University of Birmingham, Birmingham B15 2TT UK.

In the present study we assess the ability of trichloroethanol (TCE) to modulate the function of the 5-HT3 receptor expressed in rat vagus nerve measured by extracellular recording (Blakes et al. Br J Pharmacol 95: 299-299, 1988).

5-HT (10 mM-30 µM) induced depolarisations of the rat vagus nerve (EC50 = 0.97±0.13 µM, mean ± SEM, n = 6) and were antagonised by the selective 5-HT3 receptor antagonist ondansetron (20 mM, pIC50 = 9.9).

TCE (5 mM) significantly increased the potency and maximal response of 5-HT to depolarise the rat vagus nerve (5-HT; EC50 = 0.5±0.12 µM, mean ± SEM, n = 6). TCE (0.1-10 mM) significantly increased the response to a submaximal concentration of 5-HT (0.3 µM). TCE EC50µ= 2.5±0.13 (mean ± SEM, n = 3). THT (0.3 µM) induced depolarisations in the presence of TCE at concentrations above 10 µM were abolished.

The selective 5-HT3 receptor antagonist 8-pheylbiguanide (PBG) induced depolarisations of the isolated rat vagus nerve with near the same potency as 5-HT (EC50= 1.49±0.33 µM, mean ± SEM, n = 3) and these were also potentiated by TCE (EC50 = 0.14±0.02 µM, mean ± SEM, n = 3) and were also potentiated by TCE (EC50 = 0.14±0.02 µM, mean ± SEM, n = 3). This suggests that the TCE may be acting through the 5-HT3 receptor to increase the response to 5-HT.

In summary, TCE (5 mM) has potentiated the 5-HT3 receptor in the vagus nerve of the rat. This is a new action of TCE which may explain some of the clinical side effects of TCE.

Supported by the MRC.

808.4 


Although 5-HT3 receptors are present in the cortex and hippocampus, their physiological role is not clearly defined. We show here that 5-HT3 modulates the release of ACh from cortex and hippocampus of freely moving rats using the microdialysis technique. Perfusion flow rate was 3 µl/min. Twenty minutes hours after implantation with dialysis fiber, 5-hydroxy or hippocampus or cortex of male Wistar rats (250 g) released spontaneously 25.±0.1 pmol/10 min ACh (N=87), measured by HPLC with electrochemical detection. Two identical 100 nM ACh stimulations, given through the dialysis fiber at a 90 min interval, each almost doubled ACh release. 5-HT (1-50 µM) inhibited K+-evoked release of ACh up to about 50% from cortex and hippocampus. The 5-HT3 agonists phenylbiguanide (0.1-10 µM) and 1-m-i-phenylbiguanide (1-10 µM) mimicked 5-HT effect in cortex, but not in hippocampus. The effects of both 5-HT and phenylbiguanide in cortex were completely antagonized by the 5-HT3 antagonist 125I-(S)-zacopride 205-930 (0.5 µg/kg s.c.), but not by methotepin, antagonist at 5-HT1A and 5-HT2 and not at 5-HT3 receptors. Thus, 5-HT3 receptors may contribute to the role of ACh in controlling the cortical but not the hippocampal functions.

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808.1

SELECTIVE MODULATION OF TRYPHTAHNAL HYDROXYLASE AND TYROSINE HYDROXYLASE EXPRESSIONS BY S-20342 IN THE RAT BRAIN. S. Diago, D. Haissaguerre, D. P. de Lecea, A. Förstner, S. A. Pot, D. A. Nielsen, M. Linolnova. Laboratory of Clinical Studies/DICHR, NIBIA and Biological Psychiatry Branch, NIH, Bethesda, MD 20892

The effects of an antisenesce phosphothioate oligonucleotide aimed at position 123 of rat tryptophan hydroxylase mRNA (5'TGG CTG CAC TCA TGT G3') was compared to a random sequence (5'TGG CTG AAT GTA TCG 3'). The compounds were administered directly into the median raphe nucleus via an injector inserted into an indwelling guide cannula (AP -7.7, ML ±0.0, DV -7.5) in male Sprague Dawley rats. Subjects received 3 injections of 1 mol oligonucleotide in 1 μl of saline during 12 hours. Twenty-four hours after the last injection the subjects were decapitated and discrete brain regions were dissected from the tissue blocks: midbrain, dorsal striatum, dorsal striatum, hypothalamus, hippocampus, dorsal raphe nucleus, and median raphe nucleus. Tissue samples were subsequently analyzed for serotonin, dopamine, norepinephrine and 5-hydroxytryptophan content using HPLC-EC. Prior to sacrifice, food consumption was measured to determine if an antisenesce-treated subjects and open-arm entries in the elevated plus maze were decreased compared to control subjects. In these subjects tissue serotonin, 5-hydroxytryptophan, dopamine and norepinephrine content were unaltered. Injections are underway to examine other biochemical indices of serotonin function which might account for the observed behavioral differences in antisenesce-treated subjects.

808.2

EVIDENCE FOR PRESYNAPTIC COMPENSATORY CHANGES IN SEROTONERGIC NEURONS FOLLOWING DESTRUCTION WITH 5, 7-DIHYDROXY-2-METHYLPYRIDINE. D. Parey, P. Aydin, P. A. Hall, M. Lemiskow and L. Pare, NIMH and NIAAA, Bethesda, MD 20892

Following selective neurotoxin induced lesions of the nigro-striatal dopamine (DA) system, it has been found that presynaptic compensatory changes take place in the remaining populations of DA neurons sufficient to normalize the extracellular concentrations of striatal DA. The purpose of the present study was to determine whether the serotoninergic (5-HT) system has similar characteristics following neuronal destruction. Rats implanted with microdialysis guide canulae in the striatum received a single microdialysis injection of estradiol (0.5, 100, 150, or 200 μg) 5,7-dihydroxytryptamine (5,7-DHT, a selective 5-HT neuron). Two to three weeks later the animals were killed behaviorally, and then had 180 minutes of CMA-11 microdialysis probe introduced into the striatum. Twenty hours later microdialysis samples were collected at 30 minute intervals and analyzed for 5-HT content with standard HPLC-EC procedures using a microbore column. Lesions produced by the highest concentrations of 5,7-DHT (200 μg) proved to be anesthetize while lesions produced by lower concentrations of 5,7-DHT (150 and 100 μg) were anesthetize with an elevated plus-maze. Low and medium doses of 5,7-DHT failed to alter significantly extracellular levels of 5-HT in the striatum while reducing tissue levels across a variety of terminal and perikaryal regions. The highest doses of 5,7-DHT was able to increase striatal extracellular 5-HT, which was also accompanied by substantial striatal depictions of tissue 5-HT. It appears that presynaptic compensatory changes take place in 5-HT neurons following lesioning similar to those seen in the dopaminergic systems.

808.3

AN ANTISENSE OLGONUCLEOTIDE TO TRYPHTAHNAL HYDROXYLASE HAS DIFFERING EFFECTS ON INDICES OF SEROTONERGIC FUNCTION. E. S. Hall, A. Pac, D. A. Nielsen, M. Linolnova. Laboratory of Clinical Studies/DICHR, NIBIA and Biological Psychiatry Branch, NIH, Bethesda, MD 20892

The effects of an oligonucleotide phosphothioate oligonucleotide aimed at position 123 of rat tryptophan hydroxylase mRNA (5'TGG CTG CAC TCA TGT G3') was compared to a random sequence (5'TGG CTG AAT GTA TCG 3'). The compounds were administered directly into the median raphe nucleus via an injector inserted into an indwelling guide cannula (AP -7.7, ML ±0.0, DV -7.5) in male Sprague Dawley rats. Subjects received 3 injections of 1 mol oligonucleotide in 1 μl of saline during 12 hours. Twenty-four hours after the last injection the subjects were decapitated and discrete brain regions were dissected from the tissue blocks: midbrain, dorsal striatum, dorsal striatum, hypothalamus, hippocampus, dorsal raphe nucleus, and median raphe nucleus. Tissue samples were subsequently analyzed for serotonin, dopamine, norepinephrine and 5-hydroxytryptophan content using HPLC-EC. Prior to sacrifice, food consumption was measured to determine if an antisenesce-treated subjects and open-arm entries in the elevated plus maze were decreased compared to control subjects. In these subjects tissue serotonin, 5-hydroxytryptophan, dopamine and norepinephrine content were unaltered. Injections are underway to examine other biochemical indices of serotonin function which might account for the observed behavioral differences in antisenesce-treated subjects.

808.4

EVIDENCE OF ESTROGEN (E) AND PROGESTERONE (P) ON THE EXPRESSION OF TRYPTOPHAN HYDROXYLASE (TPH) mRNA IN THE RAPHÉ NUCLEUS OF NON-HUMAN PRIMATES. M. Ercolino-Thompson, N.A. Brown, C.L. Bertha. Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006

P increases prolactin secretion in E-primed female monkeys through a neural mechanism and serotonin (5HT) is a stimulatory transmitter for prolactin. This laboratory has shown that E induces progestin receptors in SHT neurons. To determine whether E or P alters the function of SHT neurons, the expression of mRNA for tryptophan hydroxylase (TPH) was examined in ovariectomized (ovx)-control, E treated (28 days) and E+P treated non-primed (14 days E + 14 days E+P) using in situ hybridization and a 248 bp TPH probe generated with RF-PCR (n=3 animals/group). Perfusion fixed midbrain sections (4μm) were hybridized at 40°C with 35S antisense cRNA directed against the 5' substrate binding domain of monkey TPH. Adjacent sections were immunostained for 5HT to confirm the location of the dorsal raphe (DR). After a final wash in 0.1X SSC at 50°C, sections were opposed to tripit sensitive film for 14 days. Densitometric analysis with graylevel thresholding was performed at 3 or 4 levels of the DR. The number of pixels exceeding background in defined areas was obtained (pixel number) and normalized by the total number of pixels counted (pixel fraction). There was a 10 fold increase in TPH mRNA signal represented by both pixel number and pixel fraction in E treated macaques compared to ovx controls (p < 0.05). Suplemental P treatment reduced TPH mRNA expression in E levels in 2/3 animals but the average TPH mRNA level in E+P treated animals remained 4 fold higher than in ovx controls. These data indicate that E induces TPH gene expression in non-human primates. The expression of E+P on TPH mRNA was variable with the average level falling between the ovx group and the E treated group. Supported by HD17292, HD18185, DK9098, RR00163

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995

A large body of evidence suggests that the serotoninergic syndrome seen in liver failure, hepatic encephalopathy (HE), is associated with an increased brain turnover of tryptophan (T). In a recent study we have shown an up-regulated and altered neuronal release of 5-HT in chronic HE as reflected by unchanged dialysate 5-HT levels in the brain of portacaval shunted (PAS) rats. In the present study we studied the frontal neocortical extracellular levels of 5-HT in HE and its main metabolite 5-HIAA and their correlations and correlation of the 5-HT system by systemic administration of the precursor amino acid L-tryptophan (L-TRP; 1 loading dose of 280 mg/kg i.p. followed 3 hours later by 5 consecutive injections of 50 mg/kg during 5 days). Brain 5-HT turnover was determined by increased 5-HT and its metabolite 5-HIAA extracellular levels, and was regulated by a MK-801-induced pyrrolorepin and increased 5-HT turnover. The L-TRP administration increased 5-HT turnover, but did not change either intracellular 5-HT or in control rats. This intracerebral function identified that the increased extracellular 5-HT levels and, hence, the increased 5-HT turnover, is a phenomenon partly due to inhibition of the brain 5-HT uptake.


Using MK-801 as a tryptophan antagonist, we have previously shown that in rats administered with MK-801 in vitro, an increase in its in vivo availability can inhibit the locomotory response to MK-801. However, the effect of MK-801 and its receptor antagonist, 5-HT2a, on locomotion in vivo has not been studied before. In the present study we tested the effect of MK-801 on locomotion in control rats and in rats treated with a 5-HT2a receptor antagonist. Our findings showed that MK-801 was able to inhibit locomotion in control rats, but not in rats treated with a 5-HT2a receptor antagonist. These results suggest that the inhibition of locomotion by MK-801 is mediated through a 5-HT2a receptor, and that the availability of MK-801 in vivo is crucial for the locomotory response to MK-801.

809.9 THE SEROTONIN 5-HT2A RECEPTOR AND 5-HT2C RECEPTOR MEDIATION OF DOPAMINE RELEASE DURING DIRECT INJECTIONS INTO THE MEDIOLATERAL PRECORTICAL CORTEX OF RATS. D. David, L. Williams, and H. H. Myer, Laboratory of Biological Psychology, Case Western Reserve University, Cleveland, Ohio 44106.

The systemic administration of selective serotonin-2 (5-HT2) receptor agonists produces a characteristic head twitch response (HTR) in rodents. The selective 5-HT2A receptor agonist, DOI, produces a HTR following bilateral injection into the medial prefrontal cortex (MPC) of male Sprague-Dawley rats. This response was dose-dependent for doses ranging from 0 to 57 nmol/g 0.35 l, and was reversed by pretreatment with ketanserin (2.5 mg/kg i.p.). The injection of L-tryptophan (1 mg/kg, i.p.) increased the size of the MPC response, and this effect was reversed by ketanserin (2.5 mg/kg, i.p.). The administration of ketanserin to rats with bilateral lesions of the lateral ventricle that had received bilateral injections of 5-HT2 receptor agonists produced a marked inhibition of the MPC response. Thus, the 5-HT2 receptors in the MPC may be involved in the regulation of dopamine release as well as other mechanisms.

809.6 SEASONAL VARIATION IN NEUROENDOCRINE AND MOOD RESPONSE TO L-TRYPTOPHAN INFUSION IN DEPRESSED PATIENTS AND HEALTHY SUBJECTS. J. E. Davidson, B. R. Mallin, C. M. McDougall, A. Vega, D. S. Charney, C. R. Heninger, L. H. Price, Dept of Psychiatry, Yale University School of Medicine, New Haven, CT 06519.

Seasonal variation of mood disorders might be related to alterations in rhythmicity of serotonin (5-HT) function. In this study we examined seasonal effects on the neurotransmitter serotonin and its metabolite 5-HIAA in 10 depressed patients and 10 healthy subjects (aged 18-25). A randomized, placebo-controlled, double-blind treatment protocol was implemented of a 400 ml infusion of 5-HT precursor L-tryptophan (L-TRP) in depressed patients and healthy subjects. Methods: Twelve drug-free patients with DSM-III-R major depression and 58 healthy comparison subjects participated. After an overnight fast, subjects received an i.v. infusion of L-TRP 2 g. Blood was obtained for serum prolactin (PRL), growth hormone (GH), and tryptophan, 5-HIAA, and serotonin (5-HT) concentration. Results: Cointer analysis revealed seasonal variation in peak change (P) PRL in the combined depressed patients (P<0.02) and in urinary (P<0.04), nonmetabolically (P<0.02), and nonphysiologically (P<0.02) subsamples, with winter acons and summer troughs; healthy subjects showed no seasonality. Peak GH showed seasonal variation in healthy subjects (April acrophase and February trough, p<0.01), but not in depressed patients. Baseline tryptophan-lp2 levels demonstrated summer peaks and winter troughs in the combined depressed group (p<0.01) and in urinary (P<0.009), nonmetabolically (P<0.02), and nonphysiologically (P<0.02) subsamples. A negative correlation was found between peak P and PRL and tryptophan levels in combined depressed (P<0.02) and urinary patients (p<0.04). Baseline GH levels also manifested seasonal variation in the combined depressed (p<0.01) and in bipolar (p<0.01), melancholic (p<0.01), nonpsychic (p<0.04), and psychic (p<0.07) patients. Conclusions: Our data are consistent with previous evidence that central 5-HT function is abnormal in depressed patients and further suggest a seasonal variability of such abnormalities that is absent in healthy subjects. Seasonal patterns of 5-HT function contribute to specific diagnostic subgroups suggesting that pathobiologically these diurnally mood-disorder subgroups may be heterogeneous.

809.8 ROLE OF NITRIC OXIDE (NO) IN PENILE EJECTION AND YAWNING INDUCED BY 5-HT2A RECEPTOR AGONIST. J. L. de Paz, L. R. Casillas, D. M. H. Aragón*, S. R. Melis, J. R. Brodie Jr., Neurosciences, Cibigleri University, 90124 Cagliari, Italy.

The effect of 5-HT2A receptor agonist (NAME) and N-monomethyl-L-arginine (NMMMA), two inhibitors of NO synthase, given into a lateral ventricile (i.c.v.) or to rats, on the development of hyperlocomotion induced by 1-(3-chlorophenyl)-piperazine (m-CPP) and N-(3-trifluoromethylphenyl)-piperazine (TFMP) in rats with 5-HT2A receptor agonists. Studies were performed in male rats. Both NAME (100, 200, and 500 μg/kg) and NMMMA (300, 500, and 1000 μg/kg) prevented dose-dependently the behavioral response induced by m-CPP (0.5 mg/kg s.c.) or TFMP (1 mg/kg s.c.) and displayed a pharmacological effect. The combination of both inhibitors was more potent than NMMMA while D-NMMA, which does not inhibit NO synthase, was ineffective. The inhibitory effect of NAME on m-CPP and TFMP responses was prevented by the co-administration of L-arginine (1 mg i.c.v.), N-CPP- and TFMP-induced penile erection and yawning was prevented also by the i.c.v. administration of L 813583 (50-200 μg) or methylene blue (50-400 μg), two inhibitors of guanylate cyclase but not by reduced hemo-lobin (50-400 μg), a NO scavenger. The results suggest that central NO is involved in m-CPP receptor agonist-induced penile erection and yawning.

809.10 VOLATOMETRIC MEASUREMENT OF SEROTONIN IN THE SUBSTANTIA NIGRA PARS RETICULATA OF FREELY MOVING RATS. J. L. González-Alfaro, F. Fernández, P. Heredia, J. L. Bata, G. Arankowsky, J. Aceves and D. Martínez-Pong, Centro de Investigación, Regional, Universidad Autónoma de Yucatan, Méjico, 97000.

The substantia nigra pars reticulata (SNR) receives serotonergic innervation from the raphe nuclei. Using different approaches, we have measured the extracellular concentration of serotonin (5-HT) in the SNR of freely moving rats. Thirty hours after implanting the CPM, an oxidation peak was recorded at an applied potential of Allopurinol (20 mg/kg. i.p.) reduced the peak half-width 55 ± 4%, indicating the contribution of uric acid (15.5 ± 4.6 Å) to the oxidation peak. Pargyline (40 mg/kg. i.p.) decreased the response on peak height to 32 ± 3%, indicating that 5-HT (2 ± 1.0 μm) is the peak. The remaining peak was considered to be 5-HT because it appeared at +307 ± 6 mV and was increased by the 5-HT reuptake blocker duxolazine. The baseline concentration of 5-HT (45.6 ± 10.8 nM) could be an overestimation due to the inhibitors of 5-HT2A metabolism. It is concluded that 5-HT is released in the SNR. (Supported by grant 1831-M9211 from CONACyT, Méjico.)
809.11


To evaluate the neurochemical dynamics of serotonergic and dopaminergic systems in post-hypoxic myoclonus, the extracellular release of serotonin (5-HT), dopamine (DA) and their metabolites were determined using in vivo microdialysis. Basal and stimulated release of 5-HT, DA and DOPAC were monitored in the prefrontal cortex of a stimulus-sensitive myoclonus model. KC (100 mM) or NMDA (500 μM) were locally infused to evoke the release of neurotransmitters. Basal levels of these neurotransmitters were unaffected among the three groups (control, post-hypoxic, post-hypoxic-recovered rats). A significant reduction (p < 0.01) in KCl- and NMDA-stimulated release of 5-HT and DA was observed between control and post-hypoxic rats. The depolarization-induced reduction in SHIA was markedly elevated in post-hypoxic rats as compared to controls. These neurochemical changes returned close to normal levels in post-hypoxic-recovered rats. A linear relationship was obtained between neurochemical alterations and behavioral quantitation of myoclonus. These data suggest that a synergistic hyperfunctioning of stimulus-induced serotonergic and dopaminergic terminals in mesocortical regions may contribute to the behavioral expression of post-hypoxic myoclonus. (Supported by Mycolous Foundation).

809.13

EFFECT OF REPEATED EXPOSURE TO FORCED SWIMMING STRESS ON EXTRACELLULAR LEVELS OF 5-HT IN THE RAT. T. Kirby* and L. Lucki, Departments of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Previous work using in vivo microdialysis demonstrated that forced swimming produces regionally-selective changes in extracellular 5-hydroxytryptamine (5-HT) in rats (Kirby et al., Brain Res., 1995, in press). Swimming produced increases in 5-HT in striatum, decreases of 5-HT in amygdala and lateral septum, and did not change 5-HT in frontal cortex and hippocampus. This study examined these neurochemical changes after repeated exposure to the swim stress. Canulae were implanted under surgical anesthesia into either striatum or lateral septum. One week later, dialysis probes were lowered through the canulae and dialysis samples collected before, during, and after a 15 min swim exposure. This dialysis procedure and swim exposure was repeated the following day. Dialysate content of 5-HT and 5-hydroxyindole-acetic acid (5-HIAA) were measured by HPLC. On the test day, swim produced an elevation of extracellular 5-HT by 60% over baseline in striatum and a reduction to 40% below baseline in lateral septum. On the second day, swim had no effect on 5-HT in either brain region. On both days, however, swim produced decreases in 5-HIAA in both regions to 40-50% below baseline. These results suggest that there is an adaptation of the regionally-specific effects of forced swimming on extracellular 5-HT, but not of 5-HIAA, over repeated exposure to the stress. Supported by USPHS grants MH 17118, MH 36522, and MH 48125.

809.14


The effect of forced swimming for 30 minutes on extracellular levels of 5-hydroxytryptamine (5-HT) and its major metabolite, 5-hydroxyindole-acetic acid (5-HIAA) were examined in the dorsal raphe nucleus, a region containing 5-HT cell bodies. Extracellular levels of 5-HT and 5-HIAA were measured using in vivo microdialysis with electrochemical detection with 10 min resolution. A dialysis probe was implanted into the dorsal raphe nucleus under surgical anesthesia on the day prior to the study. The next day, dialysate samples were collected for 70 min to establish baseline. Forced swimming initially produced a 40% decrease in raphe extracellular concentrations of 5-HT and 5-HIAA. 5-HT concentration remained suppressed for approximately 2 hours following the swim. 5-HIAA concentration remained reduced for 30 min, but rebounded to increase 20% above baseline 2 hours after the swim. This indicates that 5-HT metabolism in the dorsal raphe nucleus was likely increased while 5-HT release was reduced by the forced swim. Previous work in our laboratory has shown that forced swimming produced a regionally-selective pattern of changes in extracellular 5-HT (Kirby et al., Brain Res., 1995, in press). Since forced swimming is used as a screen for antidepressant drug effects, it is possible that regional changes in levels of 5-HT may play a selective role in mediating the behavioral effects of antidepressant drug treatments. Supported by USPHS grants MH 17118, MH 36522, and MH 48125.

809.15

PERIPHERAL AND CENTRAL INDICES OF SEROTONIN FUNCTION AND IMPULSIVITY. C. Rest*, D. Helmers, L. Albers, S.W. Tang, UC-Irvine, Psychiatry Service, VA Medical Center, Long Beach, CA, 90822.

A large body of literature exists implicating serotonin in psychiatric and behavioral disorders. There is evidence for its role in suicide, aggression and other behaviors characterized by impulsivity. Efforts continue to refine serotonergic challenge paradigms and to develop new tools to examine this system at the molecular level. In the present study korsakoff's 5-HMA, mediated intracellular calcium response to serotonin was also measured to assess signal transduction. Paroxetine elicited a robust cortical response which was directly correlated with the magnitude of platelet calcium response. Both of these measures were inversely correlated with the trait of impulsivity as measured by the Barratt Impulsivity Scale. These results suggest that paroxetine has utility in studying serotonergic systems.
**THURSDAY AM**

**OTHER NEUROTRANSMITTERS: MISCELLANEOUS**

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**810.1**

ATP-INDUCED OSCILLATIONS OF CYTOSOLIC Ca2+ ACTIVITY IN CULTURED ASTROCYTES AND IN GLIOMA CELLS, K. O. Kocrk, Institute of Neurochemistry, University of Magdeburg, Leipzig Str. 44, 39120 Magdeburg, Germany.

Continuous stimulation with ATP induced [Ca2+] oscillations (1-2/min) in cultured astrocytes from newborn rats and in rat glioma cells (C6-4-2). The initial [Ca2+] response to ATP resulted from IP3-induced Ca2+ release, whereas the subsequent oscillations were dependent both on IP3-sensitive Ca2+ stores and Ca2+ influx.

Depolarization by 50 mM [K+] resulted in a transient Ca2+ response in astrocytes, presumably due to activation of voltage-dependent Ca2+ channels. In C6-4-2 glioma cells, [Ca2+] oscillations were also induced by bradykinin. Simultaneous recording of membrane potential showed that the oscillations of [Ca2+] and of membrane potential in glioma cells were synchronous. The oscillations were affected by the K+ equilibrium potential and by blocking K+ (Ca2+) channels, indicating a potentiation of Ca2+ influx by membrane hyperpolarization. Whole-cell patch-clamp experiments indicate a conductance for Na+ and for Ca2+.

The oscillations were also influenced by hypotonic and by hypertonic medium in glioma cells as well as in astrocytes. We conclude that in glioma cells there is a feedback regulation between cell volume and [Ca2+]. The experiments indicate a possible physiological function of Ca2+ oscillations in volume regulation of glial cells.

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**810.3**

EXAMINATION OF BOVINE APO-OPSIN AS A MODEL OF LIGAND BINDING RECEPTORS, W. A. Clark, J. C. Gutierrez, and J. K. Northup, Lab of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892-4000.

Rhodopsin and the associated G-protein transducin have been utilized by numerous investigators to examine the mechanism of receptor-G-protein interaction. We have extended this work using apo-opsin to serve as a model for ligand binding seven transmembrane receptors. Bovine rhodopsin-containing rod outer segment disc membranes were depleted of chorophore using homolysis at basic pH. In an established in vitro transducin activation assay (Fawzi et al., JBC 266:12194, 1991), the resultant apo-opsin demonstrated ~50-fold lower activity than rhodopsin. Reconstitution of this opsin with 9-cis- or 13-cis-retinal in the dark results in a significant inhibition of opsin activity below basal. Upon exposure of this "inverse agonist"-bound state of opsin to light, full rhodopsin activity is achieved. Furthermore, apo-opsin responds to all trans-retinal in a dose dependent manner to form a reactivated rhodopsin species indistinguishable from native rhodopsin in the transducin activation assay. Data are presented demonstrating the effects of transducin in modulating the apparent affinity of apo for all trans-retinal. These studies utilizing opsin provide novel insights into the functioning of this class of receptor.

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**810.5**


We have recently discovered that agmatine (decarboxylated arginine), an endogenous ligand for imidazoline receptors, and its biosynthetic enzyme, arginine decarboxylase (ADC), are expressed in mammalian brain (I et al., Science 263: 966-969, 1994). In bacteria, the major metabolic pathway for agmatine is hydrolysis by agmatinase to putrescine, the precursor of polyamines, which is essential for cell viability. In mammalian brain, however, agmatine is not hydrolyzed by any known enzyme. In order to determine whether agmatine can be metabolized by a similar pathway in rat brain, agmatinase was assayed by measuring hydrolysis of guanido-C-agmatine to C-urea and putrescine and subsequent trapping of CO2 released by urease from C-urea. Incubation of guanido-C-agmatine with brain homogenates resulted in a substantial hydrolysis of agmatine (7.6 to 11.8 mmol/hg/min). Activity is reduced (up to 75%) by bovine serum albumin (25% of total activity in non-homogenate controls due to non-enzymatic degradation of agmatine. With subcellular fractionation of rat brain, agmatinase activity was maximal (48.1 nmol/hg/min) in the soluble fraction of the P2 pellet (synaptosomal/mitochondrial).

Further fractionation of the P2 pellet resulted in enrichment of agmatinase in the mitochondrial (327.8 nmol/hg/min) vs. synaptosomal (31.2 nmol/hg/min) protein fractions. Agmatinase activity in the P2 pellets varied regionally in brain: hypothalamus (133 nmol/hg/min) > hippocampus (88) > medulla (64.5) > cerebellum (47.5) > striatum (35.8) > cerebral cortex (30.2). We conclude: (a) rat brain expresses agmatinase which can convert agmatine to putrescine and urea; (b) the enzyme is soluble and associated with mitochondria; (c) the agmatine-putrescine pathway suggests a novel metabolic pathway for polyamine biosynthesis in brain.

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**810.6**

"CAGED" CARBON MONOXIDE: MOLECULES FOR PHOTO-RELEASING FREE CARBON MONOXIDE IN SITU. Joseph P. Y. Kao, D. Weinstock* & Paul F. Kesz, Medical Biotechnology Center, and Departments of Physiology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, MD 21201.

We report the design, synthesis, and application of NF-CO, NV-CO, and NF-P-O, a family of three chemosensitive reagents that release the messenger molecule carbon monoxide when illuminated with long-wavelength ultraviolet light. The caged CO's are useful as Na+ or K+ salts, which can be bath-applied or introduced into cells via patch pipettes, respectively. The caged CO's can be loaded into live cells by incubation with the acetyloxymethyl (AM) ester forms of the reagents. The light-induced "uncaging" reaction is characterized by t1/2 = 60 µs. Carbon monoxide photoreleased from intracellular caged CO is as effective as exogenous gaseous CO in activating intracellular guanylyl cyclase.

The photosensitive "cages" in these caged CO compounds are based on o-nitrobenzoyl chemistry—a miniaturized version of a methodology commonly used in chemistry. Bovyl mercury or xenon light sources are the sufficient to activate photorelease of CO. Because photorelease offers the dual advantages of spatial and temporal control of messenger molecule delivery, these caged CO's are ideal for applications in signaling studies. (Supported by GM46655 and an SRS Grant from the Univ. of Maryland School of Medicine)
A LYSINE RESIDUE OF THE CANNABINOID RECEPTOR IS CRITICAL FOR RECEPTOR RECOGNITION BY SEVERAL AGONISTS BUT NOT WIN55212-2. Z. H. Song* and T. L. Bonner, Lab. of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

Lysine192 in the third transmembrane domain of the human CB1 cannabinoid receptor was converted to an alanine to study its role in receptor recognition and activation by agonists. H-J210, CP-55940, WIN55212-2, HU-210, and anandamide were examined. WIN55212-2 and inhibition of cAMP accumulation by cannabinoid agonists were demonstrated, with different ligands exhibiting the expected rank order of potency and stereoselectivity in competition binding and functional assays. In cells expressing the mutant receptor, the binding affinity of the receptor for [3H]WIN55212-2 was only slightly affected (the K_d for the mutant receptor was twice that of the wild-type), and the ability of WIN55212-2 to inhibit cAMP accumulation was unchanged. However, H-J210, CP-55940 and anandamide were unable to compete for [3H]WIN55212-2 binding to the mutant receptor. In addition, the potencies of H-J210, CP-55940 and anandamide in inhibiting cAMP accumulation were reduced by more than 100-fold. These results demonstrate that lysine192 is critical for receptor binding by H-J210, CP-55940 and anandamide. Since lysine192 is not important for receptor binding, the interaction between WIN55212-2 and wild-type receptor is likely mediated by other amino acid residues.


Substance P (SP) typically initiates signal transduction through its binding to neurokinin 1 (NK1) receptors. SP is known to compete with the production of interleukin-1 from human monocytes; however, these cells are devoid of high affinity NK1 receptors. Jeunemaitre et al. (J Immunol, 1994, 152:2967) have recently identified a low affinity non-NK1 receptor on human monocytes. To further characterize the properties of this receptor, we have examined the binding of the non-NK1 antagonist, L-73,060, to human myeloid leukemia (THP-1) cells. The THP-1 cells were stimulated with lipopolysaccharide for 48 hr, subsequently harvested, and membrane homogenates were prepared. Binding of [3H]L-73,060 was performed at 4°C in Tris buffer, pH 7.4, containing a cocktail of protease inhibitors. Association analysis revealed that [3H]L-73,060 binding saturated within 10 to 15 min. Displacement of [3H]L-73,060 binding by SP indicated an apparent affinity of ~200 nM. Interestingly, SP did not inhibit [3H]L-73,060 binding, nor did a variety of NK peptide antagonists and agonists. To clarify these observations, additional studies were conducted as above using [35S]SP. Unlabeled SP caused a dose-dependent inhibition of [35S]SP binding, revealing a low affinity binding site. L-73,060 and the other NK compounds tested above were able to compete for [35S]SP binding.

These data provide evidence of a low affinity NK-like receptor on THP-1 cells which may have a distinct non- peptide regulatory/modulatory binding site in addition to a peptide binding site.

ZINC PROTOPORPHYRIN IX (ZnPp) INHIBITS RELAXATION AND SECOND MESSENGER ACTIVATION IN THE RAT AORTA IN A MANNER DISTINCT FROM INHIBITION OFHEME OXYGENASE (HO). Lars Ny*, Lars Grundemar, and Karl-Erik Andersson, Department of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden.

Carbon monoxide (CO), which can be produced by HO through degradation of heme has been claimed to be a neuronal messenger and a regulator of the vascular tone. We examined whether the HO inhibitor ZnPp and other porphyrins affects vasodilation evoked by various agents in the rat aorta. ZnPp (0.1 mM) virtually abolished the relaxation evoked by vasoactive intestinal peptide (VIP), atrial natriuretic peptide (ANP) and attenuated the relaxation induced by acetycholine (ACh). Furthermore, ZnPp did not affect the relaxation evoked by forskolin and 3-morpholino-sydnonimine, agents which directly activate adenylate cyclase and guanlylate cyclase, respectively. Also SnPP and PP attenuated the VIP-evoked relaxation. ZnPp also abolished the elevation of cAMP and cGMP levels evoked by VIP, ANP and ACh. Probably by interfering with membrane receptor-coupled dilatory signal transduction pathways. This effect does not seem to be due to the inhibition of HO. It appears that the lack of specificity of the HO inhibitors makes them less suitable as tools in the investigation of a messenger role of CO.

WHOLE CELL PATCH-CLAMP MEASURED ANGIOTENSIN II-INDUCED CURRENT CHANGES IN BRAIN SLICES. J.P. Mariscal, J. Abad, J.N. Thomas, and M. Nicolaides. Neurolgie des Maladies de la Caisse d'Epargne de France, 11 pl. Maresel Berthelot, 75231 Paris CEDEX 05, France. Previous experiments on anaesthetized rats have shown that many neurons in the septo-pretectal continuum are sensitive to Angiotensin II (ATII) and/or Losartan (Los).

Neuronal post-synaptic currents evoked by short time depolarizations were recorded in slice preparations of the septo-pretectal continuum from juvenile rat brains using whole cell patch-clamp techniques. The channel was blocked by a non-selective antagonist of the angiotensin Type-1 receptor, Losartan (DUP 755), was also used. 

Rats weight between 60 and 100 g on the day of the sacrifice. The temperature of the perfusion media was 35-37°C. For cell series several different graded monophasic currents (increasing by 20 nA) were applied under control conditions and then after bath application of ATII or Los. 

Preliminary results showed that at least two types of units were recorded. In the first type, the presence of ATII in the bath produced an increase in the amplitude of the mono- and bi-phasic actions potentials whereas in the second type, the cells were affected by ATII but not Los. 

The second type of unit observed showed, under control conditions, weak, burst dependant, rapidly inactivating, inward currents. These currents disappeared in the presence of ATII, and were unaffected application of Los in the bath.

These results are consistent with observations made previously in our laboratory with extra-cellular recordings and micro-iontophoresis as well as in behavioral studies. The variability of the responses to iontophoretic application of ATII as well as the long term action of Los already observed in our laboratory were quite puzzling. These, whole-cell recordings show that ATII must not be seen as a mere neurotransmitter but rather as a subtle neuromodulator capable of acting on different receptors, pre as well as postsynaptically. Los, on the other hand, has a much longer, and arguably more complex, action.

(Supported by MH 43787 and the Société Évian)
810.13  

Heme oxygenase (HO) catalyses the oxidation of heme to biliverdin and carbon monoxide (CO). Since CO can activate soluble guanylyl cyclase, it is likely that HO plays a role in cGMP mediated signal transduction. When differentiated with NGF, PC12 cells express the neuronal isoform of heme oxygenase, HO-1. HO-1 enzyme activity in cellular sonicates after 6 days of NGF treatment is 5 mol/mg/hour, which is higher than that in total brain homogenates. Also, as early as day three of NGF treatment, there is a cGMP signal by immunobioassay that is sensitive to ZnPPIX, an inhibitor of HO, but not to nitroarginine. These data suggest that as PC12 cells differentiate into their neuronal phenotype, they synthesize HO-2 which is used in cGMP mediated signal transduction. This makes PC12 cells an interesting model system in which to study the role of neuronal HO-2.

810.14  
**IMMUNOCHEMICAL LOCALIZATION OF HEME OXYGENASE-2 IN RAT BRAIN**. M. Yamanaka1, Y. Nishimura2, R. Sembel2. 1Dept. of Anatomy II, 2Dept. of Physiology II, Mie University School of Medicine, Tsu, Mie 514, Japan.

Carbon monoxide is a membrane-permeable gas that has been suggested to play a signaling role in the brain. It is formed by the enzyme heme oxygenase-2 during the conversion of heme to biliverdin. In the present study, we raised antisera against the synthesized N-terminal fragment of this enzyme and studied cellular distribution of the enzyme in the rat brain by an immunocytochemical method. Heme oxygenase-2 immunoreactivity was observed only in neurons. Though immunoreactive neurons were ubiquitous to the brain, intensely stained neurons were rich in the olfactory bulb, layers III and V of the cerebral cortex, hippocampus, locus coeruleus, pontine nucleus and cerebellum. In the pyramidal cells of the hippocampus and Purkinje and basket cells of the cerebellum, immunoreactivity was manifested in the dendrite and the perikaryon rather than in the axon terminal, suggesting the function of HO-2 as a generator of a retrograde messenger.

810.15  

Heme oxygenase-2 (HO-2) is the major carbon monoxide (CO) generating enzyme constitutively expressed in the brain. In view of the potential role of CO as a neurotransmitter the distribution of HO-2 in the peripheral nervous system was investigated by immunohistochemistry, and the immunoreactive protein was characterized by western blotting using an antisera raised against rat testicular HO-2. As neuronal cell bodies in sensory ganglia (trigeminal, mesenteral, nodose, and dorsal root ganglia), regardless of their size, exhibited intense immunoreactivity to HO-2, immunoreactivity was also confined to the perikaryon and did not extend into the axon. Similarly, all neuronal cell bodies of sympathetic ganglia (superior cervical, stellate, inferior mesenteric, and lumbar chain ganglia), and submucosal and myenteric ganglia of the small intestine of the guinea-pig were HO-2 immunoreactive. Peripheral tissues and organs so far investigated by immunohistochemistry (mucosal arteries, fat tissue, skin) were devoid of HO-2 immunoreactive axon terminals. Western blots revealed an HO-2 immunoreactive protein with an apparent molecular weight of 36 kDa in cerebellum, sympathetic and dorsal root ganglia. The present findings establish an HO-2 immunoreactive protein in the ubiquitous component of sensory and autonomic neurons in the guinea-pig. Its molecular weight corresponds to that of brain HO-2 which is 6 kDa smaller than that of testicular HO-2. The ubiquitous distribution excludes a specific association with a particular functionally defined subset of neurons. The prominent localization in the perikaryon suggests a role in the catabolism of the numerous heme proteins synthesized and utilized by peripheral neurons. (supported by the DFG, Ku 6882-2)

810.16  
**BIOLOGICAL EFFECTS OF ENDOTHELIN-1 ON ASTROCYTES ARE MEDITATED BY ETA RECEPTOR THROUGH SEVERAL G PROTEINS**. S. Gavrilov1, P. Lakomka1, A.D. Stroh1, P.O. Couraud1. 1CNRS UPR 0415, Institut Cochin de Génétique Moléculaire, and 2CNRS UA 641, Faculté de Médecine Villémur, Perpignan.

Astrocytes have been shown to express endothelin-1 (ET-1) receptors functionally coupled, via different heterotrimeric G-proteins, to several intracellular pathways at regular HO-2. It is reported here that both receptor subtypes, ETa-R and ETb-R, mRNAs were detected in primary cultures of astrocytes. To assess the relative contribution of each subtype in the astrocytic response to ET-1, effects of BQ-123, an antagonist selective for ETa-R, and IRL1620, an agonist selective for ETa-R, were investigated. Binding experiments indicated that ETa-R is the predominant subtype in these cells. Inhibition of the forskolin-stimulated cAMP production was observed under ETa-R stimulation. Shorter pertussis toxin pre-treatment completely abolished this effect, indicating that this pathway is coupled to ETa-R via Gi protein. Increase of tyrosine phosphorylation of cellular proteins, stimulation of mitogen-activated protein kinase (MAPK) and DNA synthesis were also found to be mediated by ETa-R, but through PTX-insensitive G protein. IRL1620-induced MAPK activation involved the adaptor proteins Shc and Grb2, and the serine/threonine kinase Raf-1. This study reveals that the various effects of ET-1 in astrocytes are mediated by ETa-R which couples to multiple signaling pathways via distinct G proteins.

811.1  
**STUDY OF THE FUNCTIONAL ROLE OF N-LINKED GLYCOSYLATION SITES IN GAT-1 GABA TRANSPORT AND REQUIREMENTS FOR GLYCOSYLATION IN ADDITION TO THE CANONICAL SEQUENCE**. J.A. Clark. Laboratory of Cell Biology, NIMH, Bethesda, MD 20892-4000.

Isolation of Na+/Cl- dependent transporter cDNAs has made it possible to study the structure and function of these important proteins, with the goal of attaining a better understanding of how these carriers work. The Canonical sites for N-linked glycosylation are found in the large extracellular loop that connects transmembrane domains three and four in nearly all members of this transporter family. Although the role of N-linked oligosaccharides may be trivial for the function of some proteins, the role is significant and varied in the function of many proteins. Studies have been under way to understand the role that glycosylation plays in GAT-1 GABA transporter function. Mutation of Asn residues 455, 460, and 463 to Gln residues results in the removal of GABA transport from transiently transfected CV-1 cells. The nature of this loss of transport activity will be described.

In vitro translation of a GAT-1 construct resulting in truncation of the protein at Gln residue 209 results in expression of a nonglycosylated protein. However, truncation of GAT-1 at Thr residue 285 results in a glycosylated product. These data suggest that something in addition to the canonical site is necessary for N-linked glycosylation of GAT-1.

811.2  
**COCOINE INHIBITS GABA TRANSPORT AT NEURONS IN THE DORSOLATRAL SEPTAL NUCLEUS (DLSN)**. S. Shoji and J.P. Gallagher. Department of Pharmacology, Toxicology, University of Texas Medical Branch, Galveston, TX 77555.

To investigate the action of cocaine, we applied cocaine to brain slices, in vivo, and conducted intracellular recordings from neurons in the DLSN. Brain slices were obtained from drug naive rats (NR) or chronically cocaine treated rats (CR, 15 mg/kg, IP, 2x daily for 14 Days). In NR, superfusion of cocaine produced a membrane potential hyperpolarization in only 50% of sampled neurons, while slightly prolonging the duration of evoked IPSPs (GABA-A and GABA-B). On the other hand, 100% of DLSN neurons recorded from CR exhibited a hyperpolarization to cocaine (3 μM) with a significant prolongation in the duration of IPSPs. The cocaine induced-membrane potential hyperpolarization, which is due to an activation of both potassium and chloride conductances, was TXN-insensitive and persisted in zero calcium solution. Lowering the extracellular sodium (35 mM) or chloride (74 mM) blocked the cocaine induced-hyperpolarization. Higher concentrations of the potent GABA uptake blocker NO-711 (10 μM), which itself induced a membrane potential hyperpolarization and greatly prolonged the duration of IPSPs, blocked the cocaine induced-hyperpolarization. These results suggest that cocaine inhibits GABA uptake in DLSN neurons. Moreover, in brains from CR, cocaine inhibition of GABA uptake is greatly potentiated compared to NR. This latter potentiating effect of chronic cocaine may be a cellular electrophysiological correlate of behavioral "SENSITIZATION". Supported by DA-07190.

The purposed of this study was to determine the cellular expression pattern of three GABA transporters (GATs) in the rat retina using affinity purified polyclonal antibodies directed to the C-terminus of GAT-1. GAT-1 antibody specificity was tested by preadsorption of the primary antibody with 10M G-terminal peptides of known GABA and glycine transporters. Numerous GAT-1-immunoreactive (IR) amacrine cell processes are in the proximal inner nuclear layer (INL). A few IR displaced amacrine and ganglion cell bodies are in the ganglion cell layer (GCL). GAT-1-IR processes are densely distributed to all inner plexiform layers (IPL) laminae. Weak GAT-1-IR is also present in Müller endfeet and processes in the outer retina. GAT-2-IR is localized to retinal pigment epithelium (RPE) and GAT-3-IR is expressed predominantly by Müller cells. Some GAT-3-IR cell bodies are found in the proximal INL and IR processes are densely distributed to all IPL laminae. GAT-3-IR is also present in Müller endfeet and processes in the outer retina. 

Supported by NEI EY 04667 and VA Medical Research Fund.

MAMMALIAN BRAIN-SPECIFIC L-PHOSPHOGLUCONATE DEHYDROGENASE CLONE AND ENZYME LOCALIZATION. C. F. Bennett, C. S. Wafa, J. T. Tildon, Dept. of Pediatrics, University of Maryland, Baltimore, MD 21201.


A high affinity mammalian brain-specific L-phosphoglucuronate dehydrogenase (PHG) has been cloned. PHG is a member of the Na(+)- and (Cl(-))-dependent plasma membrane transporter family that includes several neurotransmitters, amino acids, and nutrients. Previous in situ hybridization studies localized rPHG mRNA to subpopulations of dopaminergic neurons in rat brain. However, no direct morphological evidence exists about the regional and subcellular distribution of the PHG protein. Thus we used an affinity-purified antiserum antibody directed against the carboxy terminus of this transporter for the light and electron microscopic immunolocalization of the PHG protein in rat forebrain. Immunoperoxidase labeling revealed abundant, punctate PHG immunoreactivity in forebrain regions that receive dense glutamatergic innervation including the olfactory bulb, piriform cortex, hippocampal formation (HF), amygdala, caudate-putamen (CPu), thalamus, and hypothalamus. Within HF, prominent immunolabeling was observed in the inner and outer thirds of the dentate molecular layer and in strata oriens and radiatum of the CA3 and CA1 regions. Electron microscopic immunogold detection of PHG in CPu revealed prominent labeling of axon terminals forming symmetric, excitatory-type synapses with dendritic spines. Unexpectedly, the majority of gold particles were observed within the axonal cytoplasm, but not at presynaptic active zones of LPHG by reducing its extracellular concentration in synaptic and/or surrounding spaces. Supported by NIH grants NS 35253, NS 16064, MH 28334, and DA 04324, and NSF 8910963.

LEUCINE INFUSED INTO THE BRAIN BY MICRODIALYSIS COMPETES FOR THE IN VIVO LARGE NEUTRAL AMINO ACID TRANSPORTER. H. R. Zidek*, Y. Huang, C. L. Zidek, Peter Baas and J. T. Tildon, Dept. of Pediatrics, University of Maryland, Baltimore, MD 21201.

The effect of leucine on the interstitial concentration of the large neutral amino acid (L-NAA) transporters in the brain was examined using a microdialysis delivery system and HPLC analysis of the eluant. Microdialysis probes were inserted stereotaxically into the hippocampus of rats. Leucine [1-14C]-labeled with a specific activity of 100-500Ci/mol was infused into the awake, free-moving rat and the eluate from the probe was analyzed for 20 amino acids. The infusion of leucine rapidly increased the concentration of phenylalanine, tyrosine, methionine and tryptophan to higher steady state levels. A 2.4-fold increase in concentration of these amino acids was observed at 10mM leucine with minor changes in other amino acids, except glutamine which doubled in concentration. The response to leucine was concentration dependent. Inclusion of 2 mM L-phenylalanine or tyrosine in the artificial CSF (instead of leucine) increased leucine and tyrosine (or phenylalanine with 2 mM tyrosine), methionine and tryptophan. This suggests that leucine, known to accumulate in Maple Syrup Uric Disease, interferes with the uptake of the amino acid precursors for serotonin and catecholamine biosynthesis. The reduced uptake may explain some of the neurological and metabolic symptoms observed in this disease. Finally, the increase in interstitial glutamine observed in the presence of leucine is consistent with the published data of Yudkoff et al. J. Neurochem. 39: 921-927. The data suggest that leucine is an amino donor for glutamine formation. (NIH grant 15659)

The presence of a high affinity N*-dependent, synaptosomal uptake system for L-polyline (PROT) has generated interest in the possible role of this transporter in synaptic transmission. Initial reports on the cloning and distribution of PRO in the CNS, although promising, were contradictory. We developed a new assay that was complementary to the published sequence of PROT. PRO were screened against GmBank to ensure specificity and labelled with [35S]ATP for film and emulsion-based situ hybridization. Rat brains were sectioned in the frontal plane, coronal and sagittal planes and hybridized with labelled probes. Control probes consisted of sense strands from the same sequence. PROT mRNA was found to be abundant in the olfactory bulb murial cell layer, external plexiform layer neuron, olfactory bulblic and anterior olfactory nucleus. Within the thalamus, specific nuclei were labelled including the anterior dorsal, lateral posterior and lateral dorsal nucleus. In the hippocampal formation, intense labelling was seen in pyramidal neurons of CA1-4, while granule cells of the dentate gyrus were lightly labelled. Labelling was observed in cerebral cortical layers III and V of the somatosensory cortex. Intense labelling was seen in neurons of layers II-III of the entorhinal cortex. Different labelling was seen in the brainstem and spinal cord. These findings are consistent with previous observations that PROT is found in some, but not all, glomarotamatic pathways in the CNS. The localization of PRO to specific populations of excitatory neurons may provide another means to regulate excitatory neurotransmission. Supported by AG-08843, NS32501, and NSF 9310965.

LOCALIZATION OF A NEUTRAL AND BASIC AMINO ACID TRANSPORTER IN RELATION TO NITRIC OXIDE SYNTHASE IN RAT FOREBRAIN. Y.M. Pickard, A. Pusztai, J. Chao, S.B. Allison, G.L. Intenberg, Dept. of Neurology and Neuroscience and Biochemistry, Cornell University Medical College, New York, New York 10021.

The neutral and basic amino acid transporter (NBAT) is one of two known members of a novel class of proteins involved in sodium-independent transmembrane transport of amino acids. NBAT is highly expressed in renal and intestinal epithelium and has been localized to enteric neurons and to selective neurons in brainstem and spinal cord in a distribution similar to that of nitric oxide (NO). In addition, L-arginine, the precursor for NO-synthesis, is a potent NBAT substrate. Thus, to further establish the potential role of NBAT in regulation of substrate availability to NO-containing neurons, we examined the localization of a previously characterized antisense immunogold against NBAT in rat midbrain neurons immunolabeled for NO synthase (NOS) in rat forebrain. NBAT immunoreactive perikarya seen by light microscopy had many morphological similarities to the NOS-labeled cells including the presence of one or more processes. Diffuse labelling was seen in the striatum, amygdala, and deep cortical laminae, and in the striatum, electron microscopic dual labeling showed immunoreactivity associated with plasma membranes of unlabeled dendrites. Although few of these dendrites or perikarya were dually labeled for NOS, many were directly assigned to NOS-immunoreactive processes. We conclude that NBAT may be involved in neuronal uptake of amino acids, which in turn regulate the availability of arginine and other substrates to NO neurons. (Supported by grants MH00708, MH40342, and HL 18974).

CHARGE MOVEMENT ASSOCIATED WITH GLYCYNE UPTAKE. Stéphane Supplisson*, Claude Bergman, Laboratoire de Neurobiologie, Ecole Normale Superieure, 46 rue d'Ulm, 75005 Paris, France.

Relationships between the non linear capacitive current and the steady-state current associated with glycine uptake were analyzed for two glycine transporters showing 96% identity in their primary sequences (gYLT1b and gYLT1b) and expressed in Xenopus oocytes. As for several previously cloned Na+-coupled transporters, relaxation currents were recorded in response to voltage steps, in the presence of substrate which disappeared while steady-state currents developed. The relaxation currents are assumed to reflect electrical charge movement within the carrier associated with partial reactions in the transport cycle. For a given voltage step, the time course of the single exponential current relaxation (i.e. the amount of charge (Q) displaced) is the same at the onset of the pulse and on membrane repolarization; it decreases with increasing glycine concentration ("charge masking"). The Q/V relationship can be fitted by a Boltzmann equation with a mean value of 5.5 ± 0.3mV. Current relaxations show Na+ and Cl- dependence. Replacing Na+ by Choline, TEA-, or N-Methyl-Glycine decreases the amount of mobile charges by 80%. In the presence of L- Glutamate (L-Glu) which does not produce uptake, the relaxation has a mean value of 5.5±0.3mV. The mean value is unchanged while increasing the L-Glu concentration. The Q/V relationship is non-linear, as the charge/mass ratio is lower. The Q/V relationship is non-linear, as the charge/mass ratio is lower. At 20µM glycine, the ratio between the steady-state current and the amount of massed charges is independent of the voltage and found higher for gYLT1b than for gYLT1b. For the same amount of displaced charges, the rat transporter produces a 2 times larger steady-state current than the human transporter.

CHARACTERIZATION OF ASPARATE RELEASE VIA REVERSAL OF CLONED HUMAN EXCITATORY AMINO ACID TRANSPORTER SUBTYPES. A. Duvoisin, T. Rougerie, J.L. Anxolabéhère, M. Koenig, Dept. Neurologie et Neurochirurgie, Hopital Civil, University of Lyon, France and INSERM U 77, Lyon, France. The use of several cloned cDNA expressing excitatory amino acid transporter subtypes (EAAT1-3) isolated from human motor cortex (Anxolabéhère et al., Neurosci. Lett., 144, 1559, 1994) for studying the physiological roles of each transporter subtypes was involved. To this purpose, different resistance paradigms were examined to determine which conditions can trigger excess release during ischemia. Moreover, cells expressing EAAT1, EAAT2, EAAT3, or vector alone (vector) control were loaded with 3H-D-Asparte (D-AS) for 30; EAAT3 transfected cells accumulated D-AS levels greater than control cells. Release was stimulated by exposure to either: (1) combined sodium azide (As - 20mM) and isocitrate (IAA - 0.5mM), which lowers cellular (2) (g) butyric acid (GABA, 10mM), which increases intracellular Ca2+; (3) taurine (Taur), which exchanges extracellular K+ for internal K+; all these conditions occur during ischemia. Results: Activity of EAAT3 expressed stimulated D-AS release from transporter-transfected cells to the same level for all subtypes, 2-fold greater than release from vector control cells. However, activity stimulation D-AS release from EAAT1 and EAAT2 transfected cells to a larger degree over vector control cells (3-fold release from EAAT3 transfected cells (6-fold greater than vector control cells). This suggests that EAAT subtypes may be differentially regulated by H+ ions. These results indicate that the stimulus required to release glutamate via reversal of uptake may differ between cloned transporter subtypes and suggest that all transporter subtypes may not be involved in ischemia-induced glutamate release to the same extent.

INSULIN REGULATES 14C-CREATIVE UPTAKE IN GLIAL AND MUSCLE CELL LINES. J.J. Scheller and Mari D. Sapp* Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322.

Creative and its phosphorylated derivative creative phosphocholine (CP) play an important role in the maintenance of amacrine/chromatophores phosphorylated by creatine kinase to form CP, which is believed to be used for the regeneration of ATP pools. An adequate supply of creatine is required for the maintenance of CP levels. Creative is selectively accumulated from the extracellular space by a sodium-coupled high-affinity creatine transporter (C4AT) located in the cellular plasma membranes from brain, muscle, and other non-neuronal tissues. C4AT has been shown by recent cloning studies to be structurally related to the norepinephrine/GABA transporter family. Insulin has been shown to influence the metabolism of creatine and phosphorylcreatine, and rapidly alters creatine accumulation in muscle.

We have recently shown that C4AT velocity increases 5-10 fold when either C6 glioma or L6 myoblast cell lines are incubated in creatine-free medium when compared with cells grown in the presence of 5 mM creatine (McCackee et al., 1995). INS Abstracts, J. Neurochem.). Northern analysis of RNA isolated from creatine-starved and creatine-fed cells showed a change in the relative amounts of C4AT mRNAs, supporting the existence of post-translational regulatory mechanisms involved in the maintenance of intracelullar creatine levels.

To further clarify the mechanisms involved in C4AT regulation, we assessed creatine uptake in C6 and L6 cells exposed to Insulin (0.1 mM). After preincubation in serum-free medium, acute (5, 10, 30 min) insulin treatment resulted in a 40 ± 2% (± S.E.M., N=5; p < 0.002) reduction of sodium-dependent 14C-creative uptake, when compared with vehicle-treated control cells. These studies will allow further clarification of the role of receptor tyrosine kinases in the post-translational regulation of creatine transport activity. Supported by NINDS CIDA NS106151.


The gene encoding the vesicular acetylcholine transporter (VACHT) has recently been localized within the first intron of the gene encoding choline acetyltransferase (CHAT) in both somatic and mammalian, including man. The conserved structure of the CHAT/VACHT locus is unique, since both genes lie in the same transcriptional orientation and both their products are required to express the cholinergic phenotype. We have previously shown that rat VACHT is encoded by several mRNAs. Two of them share a common 5’ exon (R) with one CHAT mRNA and are therefore generated by alternative splicing of the CHAT/VACHT primary transcript. We now demonstrate that the first intron of the rat CHAT gene contains two promotors, each used to generate one VACHT mRNA. We have also identified a fifth VACHT mRNA species containing the sequences between exon R and the VACHT translation initiation codon. The existence of these five forms of VACHT mRNA with different 5’-splicing and ends can now be correlated to the size diversity of the VACHT mRNAs that we previously observed by Northern analysis.
UPTAKE AND TRANSPORTERS: MISCELLANEOUS

811.15 PROTON INTERACTIONS WITH THE ACETYLCHOLINE TRANSPORTER OF SYNCYTIAL VESICLES. M. L. Nguyen, H. J. Carlborg,* and S. M. Parsons, Department of Chemistry, University of California, Santa Barbara, CA 93106.

The acetylcholine transporter (ACHT) of synctic vesicles exchanges internally located acetylcholine (ACh) for chloride ions. The effects of proton on both faces of the ACHT were studied using purified Torpedo synctic vesicles and a hypometric lysis-rescaling technique. The rate of proton leak from acidified vesicles in the presence of ACh varied with the pH, and that it "leaks" when the internal pH is set at 5.1 and the external pH is varied, uptake of sub saturating ACh decreases toward 0 at lower external pH with an apparent pK_0 of 7.62±0.7. The major kinetic effect of higher internal pH is decreased V_m for ACh with a smaller effect on the Michaelis constant. There is no pH-dependent solvent kinetic isotope effect on the rate of ACh uptake; but, there is substantial slowing of the rate of the proton leak through the ACHT. A model is presented for binding of a proton or ACh alternately to the transporter site and a second proton to an internal allosteric activating site that mediates a leak through the ACHT by site-to-site hopping will be presented.

811.17 PRODUCTION AND CHARACTERIZATION OF ANTIFUSION PROTEIN ANTIBODIES TO THE RAT VESICULAR ACETYLCHOLINE TRANSPORTER. M.-L. Gilmore, C. J. Heilmann, A. Roghani, N. R. Nash, H. D. Rees, H. Yi, S. M. Hersch, R. H. Edwards, A. J. Levy, Department of Neurology, Emory Univ. School of Medicine, Atlanta, GA 30322 and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

A DNA for the putative rat vesicular acetylcholine transporter (VAT) has recently been cloned, but little is known about the encoded protein. We produced rabbit polyclonal antibodies to VAT in order to characterize VAT protein. A glutathione S-transferase/VAT C-terminus fusion protein was used as antigen for affinity-purification of antibodies. Western blot analysis revealed specific reactivity with bands in HeLa cells transfected with VAT cDNA and which were absent in mock transfected cells. In Western blot analysis of brain homogenates, VAT-immune reactive bands were predominant in striatum, neocortex and hippocampus, with low levels in cerebellum. Immunoreactivity was eliminated by preabsorption of the protein. Immunohistochemistry demonstrated VAT immunoreactivity in neuronal perikarya in known cholinergic cell groups, while non-cholinergic neurons were not immunoreactive. Dense VAT terminal fields and fibers were present in cholinergic projection sites, including cortex, hippocampus, basolateral amygdaloid nucleus, and many other regions of the brain. In electron micrographs, VAT C-terminus reactivity was associated with synaptic vesicles in cholinergic terminals in rat striatum and hippocampus. These studies demonstrate VAT is a selective marker of cholinergic neurons, and, specifically, cholinergic synaptic vesicles and axon terminals.

811.19 INFLUENCE OF ESTROGEN ON HIGH AFFINITY CHOLINE UPTAKE IN RAT BRAIN SECTIONS. M. L. Caspers, B. E. Deverman and M. J. Fu, Dept. of Chemistry, Univ. of Detroit Mercy, Detroit, MI 48219.

Estrogen may exert a permissive effect on several enzymes of acetylcholine metabolism. The uptake of choline is the rate-limiting step in acetylcholine biosynthesis. Overstimulated female, Fischer-344 rats (2 mo) had either 17β-estradiol (0.5 or 5 mg) or placebo pellets imbedded in the nape of their necks. Placebo and 17β-estradiol-treated animals were sacrificed after 2 or 3 weeks. 24gm frozen sections of the brains were prepared and treated with hemicholinium-3 (HC-3), a competitive inhibitor of the high-affinity choline transporter. Autoradiographic studies showed high levels of specific HC-3 binding in the caudate putamen (CP) and the nucleus accumbens. Computer-assisted densitometry indicated specific HC-3 binding was 65.5% of total HC-3 binding. An 11% (P<0.04) decrease in HC-3 binding in the CP occurred when measured with 0.5 mg 17β-estradiol pellets for 3 weeks when compared to the placebo-treated group. In rats receiving 5 mg 17β-estradiol pellets, a 16% (P<0.04) decrease in HC-3 binding in the CP was noted after 2 weeks and a 20% (P<0.02) decrease was seen after 3 weeks. (Supported by a grant from the Amer. Fed. Aging Res. and a gift from J. Rose.)

811.16 VESAMICOL BINDING STUDIES OF THE CLONED RAT VESICULAR TRANSPORTER FOR ACETYLCHOLINE. A. Roghani*, S. M. Parsons* and R. H. Edwards. 1Dept. of Neurology, UCLA, Los Angeles, CA 90024 & 2Dept. of Chemistry, UCCS, Santa Barbara, CA.

A large body of evidence has implicated acetylcholine (ACh) in the pathogenesis of neurodegenerative disorders such as Alzheimer’s disease (AD). ACh transporters are rate-limiting in the removal of synaptic vesicles from which their release is regulated in response to neural activity. This storage requires active transport from the cytoplasm. Genetic studies in the nematode C. elegans recently identified a putative vesicular ACh transporter (VAC). This transporter shows sequence homology to previously cloned vesicular amine transporters and its distribution suggests a role in cholinergic neurotransmission. In RT-PCR, we have recently isolated homologous transporters, first from Torpedo californica, and then from trout spinal cord (VTAC). (PNAS 97, 10620). The full length VTAC was expressed transiently in COS cells. After 4 days of growth for the expression of the clone we observed homogenized and high speed membrane samples prepared and used for [3H]vesamicol binding assays. Incubation of membranes with various amount of vesamicol (0 to 75 nM) under equilibrium conditions resulted in high affinity and specific binding of vesamicol. K_D=5.5 nM and B_max=12 pmol/mg protein) to membranes from the VTAC-transfected cells, but not to those transfected with vesicular amine transporter. In a separate experiment, keeping the vesamicol concentration at 1 nM, the vesamicol binding increased rapidly with time to reach maximal levels in 15 min. Repeating this experiment in the presence of ATP or CCFP did not alter the binding profile, suggesting that the vesamicol binding to VTAC did not depend on a H^+-electrochemical gradient. Furthermore, the amount of vesamicol bound to the membrane from transfected cells was significantly less when the binding was performed in the presence of ACh, but not in presence of choline or GABA. Moreover, incubation of the membrane with varying amount of vesamicol (K_D) with or without ACh from showed that, as the vesamicol concentration was raised to higher and higher values, it overcame the binding inhibition by ACh in a competitive manner. The latter result are consistent with vesamicol binding to the site of substrate recognition on the VTAC protein.

811.18 THE HIGH-AFFINITY CHOLINE TRANSPORTER IS MODULATED IN VIVO BY db-cAMP. V. Vogelsperger, N. H. Neff and M. Hadzicostantinou. M. Departments of Psychiatry, Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

The high-affinity choline transport (HACT) is considered by many researchers to be the rate determining step for the synthesis of acetylcholine (ACh). The mechanism(s) involved in regulation of the transporter are not understood. We studied the effect of cAMP-dependent pathways on HACT. db-cAMP was administered i.c.v. to mice and at various time points choline uptake, ACh synthesis and choline acetyltransferase (ChAT) activity were measured in synaptosomes from hippocampus, striatum and frontal cortex. At 1 hr db-cAMP increases choline uptake in hippocampus and frontal cortex and slightly in striatum. Choline uptake remains elevated up to 6 hours later and then returns to baseline. The change in uptake is attributed to an apparent increase in V_m and not the expression of a new transporter. Administration of okadaic acid had no effect on basal or db-cAMP activated ch uptake, while a protein kinase A inhibitor prevented the cAMP induced increase.

811.20 ELECTROGENIC Na^+ K^+ PUMP IN THE DISSOCIATED MAMMALIAN CNS NEURONS. Mitsuhiro Minokata*, Miha Fujimoto and Norio Akaike. Department of Physiology, Kyushu University Faculty of Medicine, Fukuoka 812-82, Japan.

Active Na^+ K^+ transport maintains intracellular ionic concentration and involves in the cell excitation with the distinct electrogenicity. We investigated the basic properties of the electrogenic Na^+ K^+ pump in neurons freshly dissociated from the rat neostriatum by the use of the nystatin-perforated patch technique. In current-clamp mode, ouabain (10 μM) depolarized neurons, suggesting that Na^+ K^+ pump functions well under the present experimental conditions. In the voltage-clamp mode (V기에 40 mV) with the pipette solution containing 40 μM Na^+, raising K^+P3 evoked an ouabain sensitive outward current in a concentration-dependent manner (EC50=0.74 mM) under the suppression of K^+ and voltage dependent Ca^{2+} channels. Na^+ Ca^{2+} exchange. Ti^{4+}, Ni^{2+} and Cd^{2+} also evoke the outward current in the order of EC50: Ti^{4+} K^+, Ni^{2+}>Cd^{2+}. The pump activity had slight voltage dependency and decreased in hyperpolarized membrane potential. The pump activity was temperature dependent (EC50=0.74°C). The sensitivity of ouabain was also temperature dependent, and the EC50 of ouabain was 7.07 μM at 20 °C and 1.3 μM at 30 °C. Interestingly, the pump activity was still observed even with the Na^+ free pipette solution. However, such activity reversibly diminished when Na^+ was completely removed from the external solution, suggesting that Na^+ ions are continuously leaking into the cell, resulting in activating the pump. A mitochondrial uncoupler, FCCP (1 μM), also eliminated the pump current. These results indicate that Na^+ K^+ pump functions in concert with ion dymanics across the cytoplasmic membrane.

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The use of endosomal membrane vesicles is a unique approach in the study of glucose transport across the blood-brain barrier (BBB). Membrane vesicles were isolated from bovine brain microvesicles and characterized as previously described by Sanchez del Pulgar et al. J. Biol. Chem. 267: 23951-23957, 1992). Laminar membrane vesicles showed enrichment of the enzyme gamma-glutamyl transpeptidase, while abluminal membrane vesicles exhibited System-A amino acid transport activity. The uptake of radiolabeled D-glucose was determined by rapid filtration. In the absence of an inward-directed sodium gradient, laminar and abluminal membrane vesicles showed similar initial rates of glucose uptake. However, in the presence of a sodium gradient, abluminal membrane vesicles exhibited the ability to transiently concentrate glucose above the equilibrium value (overshoot). The intravesicular glucose concentration fell back towards the equilibrium value upon dissipation of the sodium gradient. This phenomenon was not seen in laminar membrae in the presence of sodium. The addition of phloretin, a specific inhibitor of the sodium-glucose cotransporter, abolished the overshoot in abluminal membrane vesicles. Kinetic analysis of glucose transport in abluminal membrane vesicles, showed the presence of 2 distinct carriers: a high affinity, low capacity sodium-glucose cotransporter, and a passive low-affinity high capacity facilitative transporter. Laminar membrae possess only a passive facilitated carrier. These results indicate that glucose transport across the BBB is more complex than initially thought, and that the abluminal sodium-glucose transporter may be important in regulating the flux into and out of the intercellular fluid of the brain. (Supported by NIH grants NS35107 and DK42331).

811.22


We have recently demonstrated the existence of a brain-specific Na⁺-dependent inorganic phosphate (P₇) cotransporter which is constitutively expressed in neurons of the rat cerebral cortex, hippocampus and cerebellum. Characterization of Na⁺-dependent P₇ uptake in cultured fetal rat cortical neurons revealed that Na⁺ uptake is low up to DIV 11, but is fully developed after DIV 12 (when > 90% of saturable P₇ uptake is Na⁺-dependent). Na⁺-dependent P₇ uptake rates were initially increased and attained steady state more rapidly at 37 °C as compared with 22 °C or 4 °C, and were inhibited at low as compared with neutral or alkaline pH. Kinetic analyses revealed a Kₗₚ for Na⁺ of 54 ± 12.6 μM and for Na⁺ of 35 ± 4.2 μM. The Vₘₚ was 1.54 ± 0.48 umoles/mg protein. The Na⁺ sensitivity and kinetic parameters observed for neuronal P₇ transport were similar to those reported for P₇ transport in renal- derived cells and membrane vesicles². A reduction in extracellular Ca²⁺ markedly decreased (> 90%) Na⁺-dependent P₇ uptake, with a threshold for maximal P₇ import of 1-2.5 mM CaCl₂. Cultures incubated in medium where equimolar concentrations of choline were substituted for Na⁺ had lower levels of ATP and ADP and higher levels of AMP than did those incubated with Na⁺. The largest fraction of Na⁺ imported with Na⁺ was concentrated in the adenine nucleotides. Omission of glucose from the incubation medium for up to 18 hours did not affect Na⁺-dependent Na⁺ uptake. However, depletion of ATP by incubation in choline-containing medium, or with metabolic inhibitors, dramatically decreased Na⁺ import rates. These data support the hypothesis that a major function of the Na⁺- dependent P₇ transporter is the import of P₇ required for the production of high-energy compounds vital to neuronal metabolism.

SECOND MESSENGERS: KINASES

811.23

REGIONAL DISTRIBUTION OF THE Na⁺/Ca²⁺-DEPENDENT "ORPHAN" TRANSPORTERS, RXT1 AND V-7-3-2, IN THE RAT CENTRAL NERVOUS SYSTEM. J. Matzen, Z. Ado, M. Pohl, B. Giro, M. Hamon and S. E. Melendez*. INSERM U288, faculté de Médecine Pitié-Salpêtrière, 75013 PARIS, France.

Rt1 and V-7-3-2 are two members of the membrane-bound protein family which includes monoamines (dopamine, noradrenaline and serotonin) and amino acids (GABA, glycine, proline and taurine) transporters. However, the substrates of Rxt1 and V-7-3-2 have not yet been identified. Northern blot studies already demonstrated that these two 'orphans' transporters are exclusively synthesized in the CNS. Further, studies on regional distributions of mRNA encoding Rxt1 and V-7-3-2 were presently performed by means of in situ hybridization with specific [35S]cRNA antisense probes. In general, both distributions superimposed each other with high to moderate densities of Rxt1 and V-7-3-2 mRNAs in the hippocampus, caudate nucleus, olfactory bulb and limbic and spinal cord. However, differences were also noted since Rxt1 mRNA was abundant in the thalamus whereas V-7-3-2 mRNA was hardly detected in this region. Furthermore, within the substantia nigra, mRNA encoding V-7-3-2 was found exclusively in the pars reticulata whereas encoding Rxt1 was found in the pars compacta. At the hippocampal level, the pyramidal (CA1-3) and granular cell layers were found to express both mRNAs, but only Rxt1 mRNA was observed in the hilus of the dentate gyrus. Complementary immunohistochemical studies with specific polyclonal antibodies showed that the Rxt1 protein was concentrated in brain regions receiving glutamatergic and/or GABAergic inputs. At the ultrastructural level, Rxt1 was found to be associated exclusively with nerve endings. Current studies on the cellular and subcellular distributions of the V-7-3-2 protein with specific antibodies should help in further assessing the differences versus similarities between these two 'orphans' members of the same transporter family.

811.1

A NOVEL KINASE (DLK) IS EXPRESSED IN NEURONS. M. Maas*, G. Jiang*, D.J. Pink*, and L.B. Holzman. 1Department of Neurology, University of Pennsylvania, Pittsburgh, PA 15261, and 2Department of Medicine, University of Michigan, Ann Arbor, MI 48109.

DLK (dual leucine zipper bearing kinase) is a serine-threonine protein kinase in the family of mixed lineage kinase that was identified and found in brain, kidney, and ovary (Holzman et al. 1994). Non-radioactive in situ hybridization with a riboprobe generated from a PstI (1790-2660) cDNA fragment demonstrated at a level of expression of DLK in most neurons of the cerebral cortex, dentate gyrus and CA regions of hippocampus, basal ganglia and brain stem nuclei, cerebellar Purkinje cells, spinal cord and dorsal root ganglion neurons. Western blot analysis of protein from cerebral cortex, hippocampus, brain stem and spinal cord, using an antibody to the carboxy terminal domain of the fusion protein revealed a 130 kd protein corresponding to the translation of a full length cDNA. Sub-cellular fractionation of tissue from rat cerebral cortex revealed that DLK appeared to be associated with the 100,000g membrane fraction, despite lacking a transmembrane domain, and is found enriched in synaptosomes isolated from rat forebrain. In brain aggregating cultures, DLK appeared as a doublet by reducing SDS-PAGE electrophoresis. Inhibition of serine-threonine protein phosphatases by okadaic acid and sodium orthovanadate reduced its electrophoretic mobility suggesting that DLK phosphorylation is probably under the regulatory control of PPI. This results suggest that DLK is synthesized in neurons, is membrane associated, and found in synaptosomes. DLK may play a role in signal transduction at the synapse.

811.2

Role of C1 and C2 domains in the cis-fatty acid activation of protein kinase C. Felek Ebiro, Hycn Chung and Kentaro Murakami*. Department of Biochemical Pharmacology, SUNY-Buffalo, NY 14260.

Protein kinase C (PKC) has been shown to participate in the regulation of synaptic plasticity in the brain. cis-Unsaturated fatty acids (cFA) such as arachidonic acid activate PKC independently or synergistically with diacylglycerol (DAG) in vitro. Recent studies using slice preparations revealed that the synergistic activation of PKC by cFA and DAG is also operative in the hippocampus (Chen and Murakami, Neuroscience, in press). A tandem repeat of cysteine rich zinc finger-like sequence present in the C1 regulatory domain in PKC has been suggested as the DAG binding domain. However, the significance of the C2 domain, which lacks one of these cysteine rich domains is insensitive to phorbol esters and DAG. The nature of interaction of cFA with PKC and the basis for the synergistic interaction with DAG in the activation of PKC is not yet known. To address these questions, we have generated PKC isoforms in which C1 or C2 regulatory domain is deleted and expressed in COS-7 cells. Comparison of the biochemical characteristics of these mutated enzymes with wild type PKC shows that the alteration of the regulatory domain significantly affects the sensitivity to DAG. Further studies are underway to determine the role of C1 and C2 domains in synergy of cFA with DAG, translocation to the membrane and substrate specificity. (Supported by NIH MH48973)

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812.3


We have previously described a brain-specific, constitutively active carboxy-terminal fragment of PKC\( \beta \) (PKM), which has been implicated as an important element in hippocampal long-term plasticity. Levels of PKM increase in LTP (Sacktor et al., PNAS 90: 8342, 1993) and decrease in LTD (see Harabeta and Sacktor, these abstracts). Physiological experiments with chelerythrine, a specific inhibitor of the catalytic domain of PKC, provide further evidence that PKM is required for the maintenance of both LTP and LTD (ibid.). Here we present a characterization of rat brain PKM, purified with DEAE-Sepharose, heparin-Sepharose and hydroxyapatite column chromatography. PKM was isolated from all other PKC isoforms, as well as from full-length PKC\( \beta \). PKM shows catalytic activity toward a number of substrates, including Ac-pseudosubstrate peptide and histone II\( \beta \). Inhibitory properties of chelerythrine are also examined with respect to PKM. An understanding of the characteristics of PKM may lead to more precise approaches for elucidating the function of this molecule in long-term synaptic plasticity.

812.5


MAP kinase (mitogen-activated protein kinase) activation defines a kinase cascade initiated by extracellular ligands binding to particular growth-factor and G-coupled receptors. A novel component of this kinase cascade termed MEK2 (MAPKKK) kinase) has been cloned and sequenced. Two in-frame termination codons are located in the 5' untranslated region followed by an initiation methionine. The cDNA encodes a protein of 619 amino acids, corresponding to a molecular size of 69 kD.

The MEK2 protein was expressed in HEK 293 cells to determine if the MAP kinase signaling cascade. Expression of MEK2 activates MEK1 and MKK4 (both MAPKK) and ERK1 and JNK (both MAPK). Thus, MEK2 activates two distinct MAP kinase cascades: (1) MEK1->ERK1 and 2) MEK4->JNK. Both ERK and JNK activate transcription factors thereby completing the transduction of extracellular signals to the nucleus for the control of specific gene transcription.

812.7

DOMAIN ANALYSIS OF ASSOCIATIONS BETWEEN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaM(KII) SUBUNITS USING A YEAST TWO-HYBRID SYSTEM S.J. Koh*, A. Morris*, A. Hadness, and M.N. Waxham. Dept. of Neurobiol, and Anatomy, Dept. of Integrative Biology, Univ. of Texas Med. Sch. Houston, TX 77030.

CaM(KII) subunits form functionally important protein-protein interactions with other CaM(KII) subunits to form holoenzymes. We have analyzed the association of the two major neuronal isoforms, \( \alpha \) and \( \beta \) in intact cells using a yeast two-hybrid system. Expression of these subunits in mammalian cell culture has suggested, in one study (Yamashita et al., 1989 JBC, 264 p19108) that \( \beta \) subunits do not polymerize unless coexpressed with \( \alpha \). We detect \( \alpha \), \( \alpha \), \( \beta \), and \( \beta \) associations between hybrid proteins in yeast. The detection of the \( \beta \) association is supported by electron microscopy studies involving purified subunits from rat brain that have suggested that pure \( \alpha \)-containing holoenzymes and pure \( \beta \)-containing holoenzymes exist (Kanaeikit et al. 1991 JCB 155, p1049). By deletion analysis, we have confirmed that the C-terminal domain is essential and sufficient for subunit association. Moreover, associations between different domains within the same subunit, including the unique domain in the \( \beta \) subunit, are currently under investigation. Our findings suggest that the yeast two-hybrid system is a useful addition to current techniques designed to study the interactions within CaM(KII) subunits and holoenzymes.

812.4

ENDOGENOUS FACILITATION OF NORADRENALINE RELEASE FROM SYMPATHETIC NERVES THROUGH PHOSPHOLIPASE C GENERATION OF DAG, VYPEROL, AND ACTIVATION OF PROTEIN KINASE C.

H. Majewski*, A. Hoare & T.V. Murphy. Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton, Victoria 3168, Australia.

We have previously shown that inhibition of protein kinase C (Majewski & Majewski, 1989; Naunyn Schmiedeberg's Arch Physiol. 339, 48-53) or protein kinase C down-regulation (Foucat et al., 1991 Mol Cell Neurosci. 1, 95-101) attenuates high but not low frequency release of noradrenaline from sympathetic nerves, suggesting that high output noradrenaline release is maintained by endogenous activation of protein kinase C. The present study was to investigate this hypothesis further in rat atria with transmitter stores were radiolabelled with \( ^{3}H \)noradrenaline. When the atria were field-stimulated at 10 Hz for 5, 10 or 15 s, there was a progressive increase in noradrenaline release and the protein kinase C inhibitor polymyxin B (21 \( \mu \)M) inhibited noradrenaline release at the longer trains (10 and 15 s) but not the shorter (5) duration stimulation. This suggests that activation of protein kinase C increases during the stimulation train. The phospholipase C inhibitor U73122 (1 \( \mu \)M) significantly inhibited noradrenaline release at the longer trains and polymyxin B added with U73122 had no further effect suggesting that both drugs operate through the same pathway. The diacylglycerol kinase inhibitor R-59949 (1 \( \mu \)M), which prevents the breakdown of diacylglycerol, significantly elevated noradrenaline release evoked by the longer stimulations. However, in the presence of polymyxin B, R-59949 had no effect on noradrenaline release suggesting the involvement of protein kinase C. From these results we suggest that during a train of stimuli there is progressive activation of phospholipase C which results in diacylglycerol formation which in turn activates protein kinase C to facilitate noradrenaline release.

812.6

ISOZYMIC DIFFERENCES IN THE INACTIVATION OF Ca\(^{2+}\)/CALMODULIN-DEPENDENT PROTEIN KINASE II DURING AUTOOPHARCHYLYSIS. A. Husemeier, S.L. Koh, I. Antovic and M.N. Waxham. Dep. of Neurobiology and Anatomy, TDept. of Neurology, University of Texas Health Science Center, Houston, Texas 77025.

Purified forebrain Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM(kinase) undergoes a time-dependent inactivation that is sensitive to the conditions of autophosphorylation. We extend these findings by demonstrating that isoforms of CaM(kinase containing different ratios of alpha (50 kDa) and beta (60 kDa) subunits produce differential time-courses of enzyme inactivation during conditions of autophosphorylation. Purified forebrain CaM(kinase (3.1, alpha/beta) lost approximately 80% of its activity, whereas purified corebrain CaM(kinase (1.4, alpha/beta) was observed to lose less than 30% of its activity during autophosphorylation in identical conditions. This observation was extended to both cytosolic preparations and crude homogenates of forebrain and corebrain enzyme. Recombinantly expressed alpha or beta subunits of CaM(kinase undergo alterations in enzyme activity that parallel the dominant isozymic traits associated with forebrain and corebrain CaM(kinase). These data indicate that differences in the ratio of alpha and beta subunits composing the holoenzyme produces isoforms of CaM(kinase with different apparent rates of inactivation, indicating that the beta subunit is not as susceptible to autophosphorylation-associated enzyme inactivation as the alpha subunit.

812.8

NMMA RECEPTOR ACTIVATION LEADS TO PHOSPHORYLATION OF ELONGATION FACTOR-2: A POTENTIAL ROLE FOR NMDA RECEPTORS IN CONTROLLING PROTEIN SYNTHESIS. A.J. Schenck*, A. Nair*, and M. Constantine-Paton * Yale University, Department of Biology and * Rockefeller University.

We are studying NMDA receptor-stimulated protein phosphorylation in the developing tadpole neocortex, where NMDA receptor function is prominent. Using an in situ phosphorylation assay, we have previously detected 5 proteins whose phosphorylation is stimulated by NMDA receptor activation. One of these proteins, NARP-90, has an isoelectric point and molecular mass similar to that of elongation factor 2 (EF-2); which is an enzyme that catalyzes the conversion of peptide-tRNA from the A site to the P site on the ribosome during protein synthesis. Phosphorylation of EF-2 by a novel calcium and calmodulin-dependent kinase (CaM(kinase III) eliminates EF-2's ability to support translation and is associated with misreading of stop codons in some non-neuronal cells. Using antibodies specific to the phosphorylated form, we have examined whether EF-2 becomes phosphorylated when NMDA receptors are stimulated in the tadpole neocortical projection. Stimulation of the tecta with 50 \( \mu \)M NMDA + 10 \( \mu \)M glutamate (NMDA treatment) caused a minimum of 3.5-fold increase in phospho-EF-2 as detected by western blotting. Furthermore, NARP-90-90, which is NMDA sensitive, was phosphorylated by application of either 50 \( \mu \)M AP-5 + 10 \( \mu \)M glutamate or 10 \( \mu \)M glutamate alone. As with NARP-90, NMDA treatment of adult frog tecta, which under the same conditions, does not lead to EF-2 phosphorylation. Finally, western blotting of 2-dimensional gels with a pan-EF-2 antibody revealed that EF-2 comigrates with NARP-90. These data indicate that NARP-90 and EF-2 have similar properties and may be the same protein. They also suggest that NMDA receptor stimulation may transiently prevent protein synthesis which could be permissive for the transition of young synapses from an immature state to a more stable form.

PhE can facilitate lordosis in female rats pre-treated with estrogen and excites neurons in hypothalamic ventromedial nucleus (VMN) through the activation of α1-adrenergic receptors, which are coupled to the phosphoinositide second-messenger pathway. We previously showed that PhE's behavioral effect could be mimicked by TPA (Bina Res. 660:23). Further experiments using in vitro and electrophysiological approaches allow us to see if the same also applied to its neuronal action. When the activity of single units recorded from VMN in hypothalamic slices was excited by PhE, a phospholipase C (PLC) and a Ca2+ release was observed. Application of PDAc excited 8 of the 10 (81%) PhE-excited neurons at 10 μM, 3/5 at 5 μM, 2/3 at 2.5 μM and 5/8 at 1.0 μM. However, as the concentration decreased, the excitation became less steep and the latency to excitation prolonged. In contrast, applications of inactive phospholipase C (10/2 units at 10 μM, 9/2 at 5 μM and 0/3 at 1 μM) or the solvents DMSO (0/7 at 0.043% for 10 μM phospholipase C) were never successful. Despite this, examination of 12,13-diacylglycerol (10 or 20 μM) did not affect PKC. PhE excited 4/13 neurons. This responsiveness is significantly lower than the 9/12 and 6/8 from slices incubated with the inactive 4α-PtdAc (10 or 20 μM) or DMSO (0.043%). Thus, the excitatory action of PhE can be mimicked by PDAc but not by TPA, and the action appears in part to involve the activation of PKC. PDAc and TPA might differ in their effectiveness and/or selectivity of PKC isoform activation. (Supported by HHSN2024 from NINDS)

10.2.12 MUSCARINIC RECEPTOR ACTIVATION POTENTIATES LITHIUM-INDUCED DOWNREGULATION OF THE PKC SUBSTRATE MARKS IN IMMORTALIZED HIPPOCAMPAL CELLS. P. Heusch*, J. V. Coss, K. J. Robinson, S. R. Vincent. Departments of Neurological Sciences, College of Medicine, Iowa University of Medicine and Technology, Des Moines, IA.

We previously reported that chronic exposure of an immortalized hippocampal cell line (HIPHC cells) to lithium chloride (1.10 mM) produces a dose-dependent down-regulation of the PKC substrate MARKS (M yristoylated alanine-rich C kinase substrate). Additionally, it was determined that the lithium-induced reduction in MARKS protein was dependent upon the concentration of insulin, and was reversed in the presence of high insulin concentrations. In the present study we have examined the effect of muscarinic receptor activation on the expression of MARKS in insulin-treated HIPHC cells. Using [3H]JNMS binding we have determined that HIPHC cells express muscarinic receptors (Bmax = 48 fmol/mg protein) which are coupled to an IP3/DAG stimulation of [3H]inositol-loaded HIPHC cells produces a dose-dependent accumulation of inositol phosphate in the presence of lithium chloride. When HIPHC cells were exposed to lithium chloride (1.5 mM) under limiting insulin conditions, addition of 1 mM carbamol significantly potentiated the lithium-induced down-regulation of the MARKS protein. The magnitude of the MARKS protein down-regulation following carbamol stimulation was greater in the membrane-bound fraction relative to the soluble fraction. Lithium's proposed action on PKC-mediated events, such as expression of MARKS protein in brain, appears to be related to insulin concentrations and is more plausibly mediated signalling through the IP3/DAG pathway. (Supported by NIMH grant MH50105).

10.2.13 CYCLIC GMP-DEPENDENT PROTEIN KINASE TYPE II EXPRESSION IN THE RAT BRAIN: AN RT-PCR AND IN SITU HYBRIDIZATION STUDY. A. El-Hussaini, C. Bladen and S.R. Vincent. Division of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, Canada, V6Z 1Z3.

Cyclic GMP is produced in neurons through the activation of cell surface guanylyl cyclases by nitric oxide, the soluble guanylyl cyclase by nitrite oxide. Cyclic GMP can alter cellular responses by regulating many proteins including cGMP-dependent protein kinases, phosphodiesterases and ion channels. Immunohistochemical experiments have shown that cGMP-dependent protein kinase type II (cGKII) is expressed in Purkinje cells and in the basal ganglia. We have used the reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization techniques to examine the expression of cGMP-dependent protein kinase type II (cGKII) in the brain. A PCR product with the size predicted from the DNA for cGKII was detected in various regions of the brain, with highest expression in the thalamus. The amplified product of this cDNA was cloned and sequenced and shown to be cGKII. In situ hybridization with riboprobes derived from the cloned cDNA for cGKII PCR product indicated that this kinase was highly expressed in the outer layers of the cerebellar cortex and in the hippocampus, and that differences in cGMP levels could be detectable in different parts of the brain. The high expression of cGKII in the hippocampus and cerebellum suggest that this kinase is involved in the regulation of these regions. cGKII gene expression in various regions of the brain system may be mediated through cGKII, which is widely expressed in the brain.


Nitric oxide acts via its receptor, soluble guanylyl cyclase, to increase cGMP levels and regulate various proteins including cGMP-dependent phosphodiesterases, ion channels and protein kinases. Two cGMP-dependent protein kinases have been identified in the mammalian brain, Type I, in Purkinje cells and the basal ganglia, and the recently identified Type II, which is more widespread through the brain, particularly in the thalamus (El-Hussaini et al., J. Neurochem. in press). We have compared the regulation of protein phosphorylation in the cerebellum and thalamus by the NO-cGMP signal in vitro. Phosphorylation experiments were carried out in vitro on the soluble and particulate fractions of both these tissues using γ-32P-ATP, and labeled proteins were examined following separation on 5-20% SDS-PAGE and the stained group of animals was injected with 20 mg/kg 7-Nitroindazole, 1 h prior to separation of the proteins for protein kinase assay, in order to inhibit endogenous NO production in the brain. This treatment dramatically decreased expression of a soluble 46 KD protein. This appears to represent the autophosphorylation of the Type II cGMP-dependent protein kinase, since it was unaffected by Rp-8-bromo-GMP, which is an inhibitor of the Type I, but a weak agonist of the Type II kinase. In the thalamus, this autophosphorylation could be restored, and indeed, significantly enhanced, by the addition of cGMP to the phosphorylation reaction. The labeling of this protein was even greater in the presence of 8-bromo-GMP or cGMP plus IBMX. Labeling of a particulate protein of 46 KD was also reduced in both tissues following inhibition of NO synthase, and this could again be reversed by exogenous cGMP or 8-bromo-cGMP. These results indicate a role for endogenous NO in the regulation of protein phosphorylation by Type II cGMP-dependent protein kinase in the brain.
812.15
STAUROSPORINE, G0-6976, AND K-252a ENHANCED KC1-EVOKED RELEASE OF 3H-NE FROM HUMAN NEUROBLASTOMA SH-SY5Y CELLS D. Harrow, C. Rest, R. H. Maris, and W. Pettit. Section of Clinical Pharmacology, Experimental Therapeutics Branch, NIMH, and NIAAA, NIH, Bethesda, MD 20892.

To explore the role of kinases in the regulation of neurotransmitter release, we have studied the effects of various kinase activators and inhibitors on the release of noradrenaline from cultures of human neuroblastoma SH-SY5Y cells. We found that the non-specific kinase inhibitor, stauroporine (STPR), strongly enhanced the amount of 3H-NE released during a 7 min incubation with 100 mM KCl, without affecting basal release. Maximal effect required a preincubation of 1 hour and a dose of 50-70 nM. Two STPR analogues, colchicine and K-252a, similarly enhanced release. Another analogue, GF-109203X, thought to be a more specific inhibitor of Protein Kinase C (PKC), inhibited 3H-NE release at 2 μM. The PKC activator, PMA, and thymotheatin (THM), enhanced KC1-evoked release when preincubated 14 min at 100 nM and 1 μM respectively, and these effects appeared to be additive to those of STPR, G0-6976, and K-252a, suggesting that the activators and inhibitors may act via separate pathways. Moreover, downregulation of PKC isoforms α and ε by 24 hr treatment with 100 nM PMA or 1 μM THM abolished the enhancement of release by PMA or THM, but left intact the effects of STPR, G0-6976, and K-252a. Twenty-four hr treatment with 1 μM PMA, however, did abolish the enhancement induced by these agents implying that there may be a PKC-sensitive step in their mechanisms, also. Finally, since both KN-62 (10 μM), a Calmodulin kinase inhibitor, and Golgistatin (50 μM), a tyrosine kinase inhibitor, significantly inhibited KC1-evoked 3H-NE release, it appears that multiple kinases are involved in the regulation of transmitter release in this system.

SECOND MESSENGERS: KINASES

THURSDAY AM

813.1 ACTIVATION OF THE PERIPHERAL CANNABINOID RECEPTOR (CB2) INHIBITS ADENYLATE CYCLASE BAYERNICH, M.1, Avadov-Reisz, T.2, Levy, K.2, Mechanism, R.1, Bazg, J., and Vogel, Z.2. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel, Department of Natural Products, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.

Two types of cannabinoid receptors have been recently cloned. The brain cannabinoid receptor (CB1) and the peripheral cannabinoid receptor (CB2). The CB2 receptor was shown to be expressed in the HL-60 promyelocytic leukemia cell line after differentiation with TPA and it is not present in brain (Munro et al., Nature 365:61-65). Activation of CB1 by tricyclic cannabinoids or with endogenous cannabinoid ligands, was shown to inhibit adenylate cyclase (AC) activity in rat brain homogenates, in N18TG2 neuroblastoma cells, and in cells transfected with CB1. We have recently shown a stable cell line that expresses CB2. Using this cell line we found that the receptor binds a variety of tricyclic cannabinoid ligands as well as anandamides. In addition, we found that activation of CB2 leads to inhibition of AC (stimulated by forskolin, PGE2, or cholera toxin). The maximal level of inhibition was approximately 50%. The EC50 values were 3, 47, and 330 mM for H1210, H1293a, and H1293 respectively. The inhibition of AC by cannabinoids was abolished by pretreating the cells with 100 ng/ml pertussis toxin indicating that CB2 couples to the G12/G13 family of GTP binding proteins. Supported by a grant from the Forschungszentrum for Molecular Biology.

813.3 GONADAL STEROID MODIFICATIONS OF RECEPTOR-INDUCED RESPONSES IN CULTURED NEURALONAL CELLS. 1. Lopez-Coviella, R. Alatorre, & F. Hernandez-Diaz. Lab. of Neuroendocrinology, University of La Laguna Medical School, 38202 S/C de Tenerife, Spain.

The generation of secondary messengers following receptor activation may depend on receptor sensitivity, coupling of agonist-bound receptor to GTP-binding proteins, and extent of activation of effector proteins. Steroid hormones may affect each one of these steps. Incubation of GnRH prok cing neurons (GT1-7 cell line) for 48 hours with 100 nM estradiol (E2) completely suppressed norpinephrine-induced intracellular cAMP accumulation. This effect was completely reversed by a 15-minute incubation period with 100 μM prostaglandin (Pg) or prostaglandone-3S (Pgs-3S; a derivative that does not cross cell membranes), prior to norpinephrine (NE) treatment. The addition of 1 μM colchicine to the medium of E2-untreated cells, 1 hour before harvesting, produced 33% increase in basal cAMP levels. This increment did not occur in E2-treated cells. However, colchicine increased cAMP levels in the presence of E2, when cells had been stimulated with NE. Thus, Pg and Pgs-3S, colchicine all reversed the effect of E2. These results may constitute the first indication of a direct effect of ovarian steroid on the response of GnRH neurons to a neurotransmitter, and suggest that gene and non-genomic effects of gonadal steroids could alter receptor-effector coupling by different mechanisms. Data on steroid receptors and cytokinotropic proteins that may be regulating these events in this and other cell lines are presented. (Supported by DGCYT PM92-0169, GAC 92-068, & GAC 93-002)

813.2 TRANSMEMBRANE SIGNAL TRANSDUCTION SYSTEMS MEDIATING CHOLINERGIC REM SLEEP GENERATION. ML. Caputo, MA. Fitzgerald, EA. Redish, and R. Liddle. Department of Anesthesiology, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

The cholinergic regulation of REM sleep has been studied in detail, yet the complex signal transduction pathway(s) responsible for REM sleep generation are unknown. Considerable data show that muscarinic receptors in the medial pontine reticular formation and the locus coeruleus are important for REM sleep generation (Saper et al., Sleep, 5/163, 1994). Most recently, Gi and Gs-like G-proteins have been shown to act as transducers in the ability of mPRF carbachol administration to cause a REM sleep-like state (Am. J. Physiol. 269(in press), 1995). As an initial step toward identifying the second messenger involved in REM sleep generation, the present study is testing the hypothesis that increasing cAMP in the mPRF will block cholinergic REM sleep generation. Cats (N=3) were implanted with standard electrodes for measuring states of waking, non-REM and REM sleep and with guide tubes for mPRF drug administration. Polygraphic recordings were made for 2 hrs following mPRF microinjection of saline (control, n=13); carbachol (8.7mM, n=12); forskolin, an activator of adenyly cyclase and a stimulator of cAMP (7mM, n=8); carbachol following forskolin pretreatment (n=9); dibutyryl cAMP, a cAMP analog (7nM, n=8); and carbachol following dibutyryl cAMP pretreatment (n=6). After all microinjections of forskolin, 2 hr recordings were made (n=17) and 48 hr (n=17) post-injection and 24 hr (n=12) after dibutyryl cAMP. The carbachol-induced REM sleep-like state was significantly (p<0.05) decreased by mPRF pretreatment with forskolin (-34.7%) and dibutyryl cAMP (-49.5%). In contrast, forskolin alone and dibutyryl cAMP alone had no immediate or long term effect on natural REM sleep. These data suggest that cholinergic REM sleep generation is mediated, in part, by pontine adenylyl cyclase and cAMP. Support: MH-4566 (HAB); HL-40681 (KE); Department of Anatomy and Neuroscience & Anatomy.

813.4 CHRONIC ANTIDEPRESSANTS TREATMENT ENHANCE ROLIPRAME INDUCED BEHAVIORS IN RATS H. Owase*, H. Kamada1, M. Yamamoto2, N. Anemuy, S. Hanai3, Sato4, H. Ohshika2, and N. Takahata1. Dept. of 'Neuropsychiatry and Pharmacology, Sapporo Medical University, School of Medicine, Sapporo 060, Japan.

It has been reported that treatment with rolipram, a selective CAMP phosphodiesterase inhibitor which reflects the enhanced availability of cAMP in the brain, results in an extraordinary behavioral syndrome characterized by head twitches (HT), forepaw shaking (FS), grooming and hypothermia in the rat. These particular behavioral alterations in rats induced by rolipram contribute to the enhanced availability of cerebral cAMP. The goal of the present study is to examine the connection between the second messenger system and animal behaviors induced by psychotherapeutic drugs. Sprague-dawley male rats were injected (i.p) once daily for 1-2 days with saline and amitripyline (10mg/kg), chlorpromazine (10mg/kg), clomipramine (10mg/kg), milnacipran (20mg/kg), fluoxetine (10mg/kg), fluvoxamine (25mg/kg), trazodone (20mg/kg), chlorpromazmine (10mg/kg) or lithium (31mg/kg). The number of rolipram-induced HT, FS and Gr actions were counted 15min after rolipram injection for 45 min. Rectal temperature was measured immediately before and 50 min. after rolipram administration. Administration of chronic (14-21days) but not acute tricyclic antidepressants and atypical (trazodone) or novel antidepressants (SSRIs or mcinacipran) augmented rolipram-induced behaviors (especially HT). Contrary to the effects of antidepressants, chronic chlorpromazmine or lithium administration decreased those behaviors. These findings provide symptomatic documentation that elevation of the cAMP cascade system may have an important role in antidepressive effects.
813.5 FUNCTIONAL CHARACTERIZATION OF A2b ADENOSINE RECEPTORS EXPRESSED BY HUMAN NONPENTIGMENTED EPIPHYLIAL (NPE) CELLS S. Michel, M. Badoud, F. Terra, and Michael W. Martin. Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107.

Activation of A1 and A2 adenosine (ADO) receptors results in either inhibition or stimulation of cyclic AMP (cAMP) activity in various cell types. In the present study, we characterized the AC responses of human NPE cells to a series of ADO receptor agonists and antagonists. Accumulation of cyclic AMP (cAMP) was determined by measuring the conversion of 3H-ATP to 3H-cAMP in pre-labelling cells with 3H-adenine. Cells incubated in serum-free media had low levels of cAMP and the PDE inhibitor RO20-1724 increased these levels only marginally. Addition of the isoproterenol (ISO) or the receptor-independent activator forskolin (FSK) markedly elevated cAMP levels. No evidence for A1 receptor-mediated inhibition of basal, ISO-, or FSK-stimulated AC activity was observed. In fact, the AI-selective agonist 6-chloro-2',3'-cyclic ADP-ribose stimulated AC activity at concentrations >10 μM in the presence of 1 μM FSK synergistically activated cAMP accumulation, even at nanomolar concentrations. Adenosine stimulated cAMP accumulation but with relatively low potency (EC50 = 34 μM). The rank order of potency of other receptor agonists was N6CA > R-JP1A > GGS21680. 7-β-arylcarboxamide adenosine (NECA)-stimulated (3H) cAMP accumulation was competitively antagonized by both xanthine (8-β-cyclopentyl-1,3-dipropylxanthine [CPX]) and 8-β-(3-thioisobutyryl)caffeine (ICTC) and non-adenosine (CGS 15943 A) agonists. The rank order of potency was CGS > NECA > CPX. These data indicate that NPE cells express a low affinity A2b receptor subtype. (Supported by NIAAA-A0689 and UNTHSC Grants).

813.7 MODULATION OF STRIATAL C-FOS EXPRESSION BY DIAZEPAM. L.P. Niles*, J.L. Smith and G.C. Tents. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3G5.

Diazepam, a non-selective benzodiazepine (BZ) receptor antagonist, inhibits cyclic AMP (cAMP) production, particularly in the rat striatum, via a putative Gβγ-linked mechanism. Since phosphorylation of a cAMP response element binding protein (CREB), by a BZ-receptor protein kinase (PKA), results in c-fos activation, the effect of diazepam on the expression of this immediate early gene was examined. Male Sprague Dawley rats (about 150-250 g) were maintained for at least one week under a 12:12LD lighting cycle with lights on from 7 am to 7 pm. They were randomly assigned to groups and injected with vehicle or drugs 4-5 hours after lights on. Two hours later, animals were killed and striata dissected on ice and frozen overnight at -20°C. Subsequently, strata were homogenized and centrifuged (containing 50 mM Hepes and nuclear fractions isolated by centrifugation. Proteins were separated by SDS/PAGE following by Western blot analysis using a polyclonal anti-cAMP antibody. Following incubation with an [3H]cAMP-labeled second antibody, cAMP-related proteins were detected by chemiluminescent autoradiography.

Preliminary studies indicate that diazepam (5 mg/kg, p.o.) induces c-fos expression while administered alone. However, pretreatment with diazepam (5 mg/kg, p.o) caused a significant attenuation of the effect of amphetamine (5 mg/kg, p.o), which by itself is a potent c-fos inducer. The actions and mechanisms underling the modulation effects of diazepam on neuronal activation, as indicated by c-fos induction, await clarification. (Supported by a NSERC Strategic grant).

813.8 IN VITRO INHIBITION OF CA2+-CALMODULIN STIMULATION OF TYPE I ADENYLYL CYCLASE BY SOMATOSTATIN. Mark D. Nielson, Enrique C. Villacres*, and Daniel R. Storm. Department of Pharmacology, University of Washington, Seattle, WA 98195.

In vitro evidence has demonstrated that the type I Ca2+/calmodulin-sensitive adenyl cyclase (I-AC) is inhibited by both the α1 and β1 subunits of guanine-nucleotide-binding proteins (G proteins). However, in vivo evidence concerning the ability of extracellular hormones to inhibit the Ca2+/calmodulin stimulation of I-AC is lacking. Here, we describe concentration-dependent inhibition of Ca2+/calmodulin stimulation of I-AC in vivo by the tetrapeptide hormone somatostatin. Treatment of human embryonic kidney 293 (HEK 293) cells stably expressing I-AC with the agonistic amino acids, carbobolch or the Ca2+/calmodulin ionophore A23187 resulted in 30-20 fold increases in intracellular cAMP levels, respectively. Utilizing the endogenously expressed somatostatin receptors expressed in HEK 293 cells, we found that somatostatin inhibited these cAMP increases 40-50%. Forskolin stimulation of I-AC was also inhibited by a similar amount. Maximal inhibition of I-AC occurred at approximately 200 nM somatostatin, with an IC50 of ~25 nM. Additionally, inhibition of Ca2+/calmodulin stimulation of I-AC was prevented by overnight pretreatment with pertussis toxin, suggesting that G-proteins of the Go/α class are involved. These results demonstrate that extracellular hormones are capable of inhibiting Ca2+/calmodulin stimulation of I-AC via inhibitory G proteins in vivo.

813.10 ACETYLCHOLINESSTERASE MODULATES DOPAMINE-STEMULATED CYCLIC AMP ACCUMULATION IN APLYSIA GILL. Srijan, M. Faller, L. Z., Jackson, B. A. and Fetter, R. Dept. of Physiology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

Acetylcholinesterase (AChE) has functions in addition to acetylcholine (ACh) hydrolysis as supported by studies of AChE regulating dopaminergic receptor responsiveness in substantia nigra (Greenfield, S.A., 1985). Recent studies have demonstrated that AChE inhibition affects non-cholinergic components of the sphen-gill ventilatory reflex in Aplysia that enhances muscle contraction in gill apparently by elevating cyclic AMP (cAMP) levels (Rüben et al., 1979). AChE is widely present in Aplysia gill and is released when gill is exposed to DA suggesting a non-cholinergic interaction between DA and AChE gill. The aim of the present study was to determine if AChE inhibition in the gill affects DA-dependent AChE accumulation, as measured by the DA dose-dependent increase in cAMP levels in isolated gill pinnales incubated in Aplysia saline. Incubation in the presence of AChE inhibitor BW28A5L, which binds to both the catalytic site and a peripheral anionic site of AChE, dose-dependently decreased cAMP accumulation in response to a maximum stimulatory dose (NmM) of DA. Total inhibition of AChE with 100μM BW28A5L reduced DA-stimulated cAMP levels by 68%. In contrast, total inhibition of AChE with 1 mM edrophonium chloride, which binds only to the catalytic site of AChE, did not reduce DA stimulated cAMP levels. Also, incubation in bath carbobolch (10 μM) did not affect cAMP levels in pinnales. These results show that AChE modulates DA-dependent AChE accumulation in Aplysia gill through a non-cholinergic mechanism involving its peripheral site. AChE's modulation of DA's effects in Aplysia gill and nigan neuroins in higher vertebrates suggests that this function of AChE is conserved across species.
813.11
CAMP IMAGING REVEALS GLUTAMATE RECEPTOR-RECEPTOR-MEDIATED REDUCTION OF [cAMP] IN PRIMARY CULTURES OF HIPPOCAMPAL NEURONS. J. E. H. Y. G. Kim** and R. E. Tamás*. 1Department of Pharmacology and 2Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA 92093-0642.

The role of cAMP in neuronal plasticity in the hippocampus is receiving considerable attention. However, traditional radio-immunoassays measure total cAMP at a single time point, usually in the presence of phosphodiesterase inhibitors, as homogenates of brain which contain neurotransmitters, astroglia, and other cell types. Using a fluorescently labeled cAMP-dependent protein kinase (CIPKR), cytosolic [cAMP] was measured in morphologically identified neurons in primary cultures of rat hippocampal cells in real time. Individual neurons were microinjected with FICRhR and stimulated with a variety of glutamate receptor agonists. Resting [cAMP] was high enough to cause partial activation of the kinase, and rapidly decreased to negligible levels at 10 sec with 50 μM AMPA. CIPKR with Kd 0.04 μM showed no response to AMPA and its effects were mimicked in FICRhR by AMPA, but not by NMDA. An estimate of E0.5 was 10 μM. Here we report that cAMP, directly or indirectly, is a neuronal plasticity factor in the hippocampus. The results are consistent with previous studies indicating that cAMP in hippocampal neurons may have a role in regulating neuronal plasticity.

813.12
ENDOGENOUS PROTEIN KINASE A INHIBITOR (PKI) MODULATES SYNAPTIC ACTIVITY. I. de Leersum, P. J. C. van den Bosch, S. Y. Beggs, I. J. M. J. Stienstra, J. S. Richter, and J. J. H. de Koning. 1Department of Pharmacology and 2Department of Neurophysiology The J. van der Hoeven Institute, University of Nijmegen, 6525 SN Nijmegen, The Netherlands.

Protein kinase A (PKA) has been shown to be involved in major regulatory mechanisms underlying synaptic plasticity and complex behaviors such as learning and memory. PKI has been extensively used as an extremely specific and potent inhibitor of PKA and the PKA-mediated signal transduction. Clear functions in 'in vivo' for PKI, however, remain to be established. Here we report that several forms of synaptic stimulation in the rat hippocampus cause a 50% decrease in the concentration of PKI. Furthermore, inhibition of antiserol oligonucleotides against PKI into the rat brain results in dramatic changes in the electrophysiology of hippocampal neurons, including the blocking of long-term potentiation suggesting a stimulus-dependent regulatory role for PKI in PKA signal transduction.

813.13

DARPP-32 (D-32) and inhibitor-1 (D-1) are homologous inhibitors of protein kinase A in the brain. Their ability to function as inhibitors is dependent on phosphorylation of threonine 34 and threonine 35 of D-32 and 1-1, respectively, by cAMP-dependent protein kinase (PKA). For D-32, which is highly enriched in dopaminergic neurons of the basal ganglia, this activated state is modulated through phosphorylation at additional sites by calcium-activated kinases I and II. Here, we report that in vitro, D-32 and 1-1 serve as substrates for phosphorylation by the cell-cycle-dependent kinase cdc2 as well as the neuronal cdc2-like kinase Cdk 5. The stoichiometries of phosphorylation by cdc2 purified from sea star oocytes have been determined for D-32 and 1-1. Two-dimensional phosphoprotein gels of D-32 and Cdc2 revealed distinct maps for each. An amino acid analysis identified the residues phosphorylated as threonine for D-32 and serine for 1-1. Sequence analysis of phosphopeptides purified by HPLC used to identify the phosphorylation sites and direct the generation of phosphorylation-state-specific antibodies. The antibodies are being used to evaluate the state of phosphorylation of these sites in tissue slices. Neuronal cdc5 purified from bovine brain appears to phosphorylate the same site as cdc2 based on phosphopeptide mapping. The expression of various cdk in asialotic tissue was shown to be developmentally regulated by immunoblot analysis. Notably, of the kinase studied, only cdc5 was detected in adult rat striatal tissue.

813.14

The mRNAs for the two known isoforms of the cAMP-dependent protein kinase inhibitor (PKI) protein (PKIa and PKIb) are known to have a differential distribution in developing rat tissues, particularly in brain and testis. As these tissues are heterogeneous in cell type, we have investigated the cellular expression of PKIa and PKIb mRNAs in developing rat brains using a specific in situ hybridisation technique. Male Wistar rats were sacrificed at day 5 intervals, beginning at day 3 of age and ending at day 60. Whole brains were removed, hemisected along the sagittal plane and rapidly frozen on dry ice. 10 μm sections were hybridised with cRNA probes complementary to the mRNAs encoding PKIa and PKIb. Film autoradiography showed high expression of PKI mRNA in cerebellar and hippocampal tissue from day 15 to 60. In in situ hybridisation (d5) PKI mRNA expression was high in individual cortical and hippocampal neurons. In adult PKIb mRNA was apparent in cortical, hippocampal, and cerebellar granular neurons. This expression was detectable from day 15, increased to a peak by day 25 and remained constant until day 60. No specific hybridisation was detectable in cerebellum or hippocampus before day 20 or in sections hybridised with sense cRNA probes. PKI was also localised in cerebellar granule cells and hippocampus in adult rat brain. PKI has been shown to be of importance in the maintenance of long term potentiation and response of hippocampal neurones to anoxia, suggesting that PKI may play an important role in the function of the hippocampus.

813.15

Previous cyclic AMP-dependent potentiation of a 35 KD nucleus accumbens protein was reduced at 32 days after bilateral 6-hydroxydopamine lesions in the nucleus accumbens. Two-dimensional gel electrophoretic analyses verified that a 35 and a 56 KD protein showed a significant decrease in cyclic AMP-dependent phosphorylation in vivo. Also a protein with a molecular weight of 45 KD showed a significant decrease in the dopamine-depleted nucleus accumbens. These results indicate an increase in the phosphoryproteins with molecular weights of 35 and 56 KD as well as a decrease in the phosphoryproteins with Kd 0.04 μM. An estimate of E0.5 was 10 μM. Here we report that cAMP, directly or indirectly, is a neuronal plasticity factor in the hippocampus. The results are consistent with previous studies indicating that cAMP in hippocampal neurons may have a role in regulating neuronal plasticity.

813.16
ISOLATION AND CHARACTERIZATION OF cAMP-SPECIFIC, ROLIPRAM-SENSITIVE, PHOSPHODIESTERASE (PDE) IV FROM PIG BRAIN CYTOSOL. Gustave Palmié and Herbert H. Schoeller** Research Laboratories, Schoering AG, 13342 Berlin, Germany.

Rolipram is a selective and stereospecific inhibitor of type IV PDE. Using a rapid and specific purification procedure we have purified the cytosolic form of a rolipram sensitive "low Km" cAMP PDE from pig brain cortex. The enzyme was isolated by affinity chromatography on 4′-aminobenzyl-Sepharose and monoisolated by MonoQ and Superose chromatography. The purification was approx. 5000-fold at 1% yield based on [H]cAMP binding capacity. The protein had a native MW of 180 KDa and a denatured MW of 90 KDa, suggesting a dimeric structure. Km (0.2 mM) and nM near 1 of H-rolipram binding were similar throughout purification steps in the presence of a factor derived from bovine tissue. In its absence Phosphorylated decreased drastically with unchanged Km and nM. It is a thermostable, lipophilic and Mg2+-dependent protein which modulates the binding of [H]rolipram in the protein in a negative or positive manner. The sensitivity of the PDE activity to inhibition by (−)rolipram improved from 300 nM (I50) in crude brain extract to 0.7 nM in the purified preparation. It decreased further to 0.15 nM in the presence of 1 μM rolipram, suggesting a dimeric structure. PKI (0.2 μM) and 20 μM dibutyryl cAMP were active with IC50s of 740, 140, 33 and 5. The endogenous enzyme inhibited cAMP metabolism significantly only at 10μM. Between 0.1-10μM, cGMP neither inhibited nor activated the purified enzyme. Calcium ion stimulated PDE activity in crude extracts 2-fold, no effect was seen in MonoQ fractions. The isolated protein revealed complex enzyme kinetic behavior. Depending on substrate concentration Kd and Vmax values varied between 2.8 and 7.3 μM and 1500000 μmol/min/mg. The type of enzyme inhibition by (−) rolipram was of a hyperbolic mixed type, probably because of the endogenous mixture of PDE IV isoforms.
18.4. MODULATION OF NMDA RESPONSES BY INTERFERON-α IN THE PREOPTIC ANTERIOR HYPOTHALAMUS. S. Takei, T. Kanaiishi, S. Tan and T. Horii, Dept. of Physiology, Faculty of Medicine, Kyushu University, Fukuoka 812-85, Japan.

The brain and the immune system share the ligand-receptor systems which enable them to communicate with each other. Interferon-α (IFN-α) is one of such ligands that are produced in both body systems and is known to induce variety of physiological symptoms. The preoptic anterior hypothalamus (POA) is one of the sites of action of central IFN-α. We previously reported that IFN-α’s modulatory effect on glutamate- and NMDA-induced whole cell currents was partially blocked by preincubation with IFN-α. Now we investigated the mechanisms of IFN-α-induced suppression of NMDA responses in the POA neurons voltage-clamped at -60 mV using 125μM halothane slices of 10-20 days old WKA rat. Intravenous injection of preincubated POA neurons with IFN-α (100-200μU/ml, 2 min) with a few minutes latency for more than one hour. This IFN-α-induced suppression of NMDA responses was partially blocked by the extracellular injection of sodium ions (10μM), and the bath application of (Tyr-D-Ala-Gly-ol-N-Me-Phe)-Gly-ol (DAGO, 2μM), a receptor selective opioid agonist, suppressed NMDA currents. Although bath application of sodium salicylate (10μM) reversed suppression of NMDA currents, prostanoid E2 (2μM) did not affect NMDA responses. Concurrent application of superoxide dismutase (SOD, 200μM) with IFN-α almost completely abolished IFN-α’s action on NMDA currents and hydrogen peroxide (1mM) mimicked it, suggesting the involvement of reactive oxygen intermediates (ROIs). Neither SOD nor SOD affected DAGO-induced suppression of NMDA currents. Neurino-L-ariginine (100μM) slightly attenuated the suppression of NMDA currents, suggesting the involvement of nitric oxide which is known to interact with ROIs. These results suggest that opioid receptor mechanism and ROIs are independently involved in IFN-α-induced suppression of NMDA currents in the POA neurons.

18.5. LOCAL LYMPH NODE APPLICATION OF 6-FLUOROXYDOPAMINE (6-OHDA) DEACTIVATES LYMPH NODE ORGANSPERINHUMAN INTERACTION. S. Mohri, M. Masuda, K. Yamanaka, M. Ishikawa, K. Kinoshita, T. Okada, T. Taniguchi, Y. Yamasaki, N. Yamasaki, K. Mita, Y. Lezono and T. Izumii, Dept. of Pharmacology and Anesthesiology, Univ. of Occup & Envir. Health, School of Medicine, Kitakyushu 807, Japan.

Interferons (IFNs) are a group of molecules that may have immunoregulatory functions. Recent studies have reported that several neurological side effects are observed in IFNα-treated patients. To delineate whether IFNα can modulate the neurosecretory functions, the effects of two IFNs, human leukocyte IFN-α and recombinant human IFN-γ, were studied in cultured bovine adrenal chromaffin cells. Treatment of cultured cells with IFN-α (1000U/ml) for 48 hr increased an accumulation of catecholamines in the cultured medium, but not with IFN-γ (1000U/ml). The IFN-α-induced response was observed in time (6-48 hr)- and concentration (10-1000 U/ml)-dependent manners. The stimulatory effect of IFN-α was not inhibited by protein kinase C inhibitor (8-7) and NO synthase inhibitor (L-NMMA), both of them were previously reported as the second messenger in catecholamine secretion. These results suggest a possibility that IFN-α acts as a neuromodulator during immune responses.

18.6. SYMPATHETIC NORDADERENERGIC INNERRATION OF SPLEEN, THYMUS AND MESENTERIC LYMPH NODES (MLN); A COMPARISON BETWEEN SPRAIGE/DAYLEW, LEWIS AND FISCHER 344 RATS. Sen. I. Shimomura, A. S. D. Feller and D. F. Felt.*, Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Primary and secondary lymphatic organs in rodents are sympathetic postganglionic noradrenergic (NA) nerve fibers. We have shown that the NA nerves in the spleens of Fischer 344 (F344) male rats, at the age of 20 weeks, increases as an age-related decline in cell-mediated immunity. The histocompatible inbred Lewis (LEW) strain is susceptible to autoimmune disorders and shows exaggerated autoimmune reaction. In the present study we compare the NA innervation of spleen, thymus and MLNs in young adult, male and female, F344, LEW and mixed strain, Wistar-Duarte (SD) rats in order to elucidate the regional and species-specific differences in their NA innervation and the potential role of the sympathetic nervous system in their immunity. All rats were subjected to identical surgical procedures and maintained in a constant environmental condition throughout the experiments. Both spleen and thymus were observed for the presence of NA fibers. The results demonstrated that there were no differences between the two strains in the spleens of the three strains was similar and followed the compartmentation reported previously. The density of innervation correlated well with the histological analysis (SD vs. LEW). Spleen and thymus in F344 rats did not differ from those in LEW rats. The difference was found in the MLN, the NE concentration in the SD strain is higher than in F344 for total NE content. This study demonstrates that male F344 rats have the most innervated splenocytes, the lowest NE concentration in spleen and MLN, and the lowest NE content in the lymph organs of male F344 rats. Our finding in the spleen is consistent with the results of others who interpreted aging studies in rodents, where the male F344 rat is the single best available model at present. The more dense NE innervation of secondary lymphatic organs in LEW rats may be important in the autoimmunity reactions (Supported by R37 MH 42706 and a Markey Charitable Trust Award).
814.7
NGF MODULATES SYMPATHETIC INNERVATION OF LYMPHOID TISSUES. Sonia J. Carreno*, Kathryn M. Allbritton*, David I. Betts*, Mark Parish, James M. Connor* & Brian M. Davis*. Dept. of Anatomy and Neurobiology & Dept. of Pathology, Univ. of Kentucky College of Medicine, Lexington, KY 40536-0884 and Dept. of Biology, Univ. of California, San Diego, CA 92093-0009.

Immune tissues are known to be innervated by the sympathetic nervous system, but little is known of what directs the innervation to specific tissue compartments. We have examined the sympathetic innervation of immune tissues in transgenic mice that overexpress nerve growth factor (NGF) in skin and other epithelial structures. NGF transgenic mice exhibited dramatic hyperinnervation in the splenic marginal zone, and the medulla and capsule of peripheral lymph nodes. In contrast, the transected mesenteric lymph nodes showed no hypertropeinnervation. This difference correlated with the location of these nodes; peripheral lymph nodes drain skin where the transgene was expressed while mesenteric lymph nodes drain non-epithelial expressing structures. In addition, the level of innervation correlated with the level of NGF peptide content as assayed by ELISA and immunocytochemistry. RT-PCR showed that the NGF transgene was not being expressed in the immune tissues, suggesting that immune tissues can concentrate transgenic-produced NGF. In addition, the change in innervation had functional consequences. The mitogen response to concanavalin A (Con A) by spleen cells was decreased in the transgenics suggesting that elevated catecholamines or NGF can modulate the proliferative response of these cells. These mice demonstrate that NGF can modulate the sympathetic innervation and function of the immune system. Supported by MH48644 to SLC and NS31826 to BMD & RMA.

814.9
BOTH BRAIN AND LYMPHOCYTE DERIVED β-ENDOPHIN EXERT IMMUNOSUPPRESSION. P. Sagare**, R. Manfredi and A. E. Panari*. Dept. of Pharmacology, University of Milan, Milano, Italy.

We previously showed that the opioid receptor antagonists naloxone and naltrindole enhance the proliferative responses of human and rat lymphocytes (1). In the present study we wanted to evaluate the role of the central, pituitary and immune-derived β-endorphin (BE) in the in vitro inhibitory effect of the peptide on splenocyte proliferation in the rat. The intraperitoneal injection of the BE (1, 5,10 ng/100 g) or the administration of BE (1, 10 ng/ml) induced a significant inhibition of PHA induced proliferation, while the iv administration of an anti BE antisemun potenitally the response. Also the intraperitoneal injection of the BE (100 μg/100 g) induced an increase of splenocyte proliferation. Since the pituitary can be considered the major source of circulating BE, in order to verify the effect of the central BE we evaluated the effect of the administration of the i.v. antiserum in hypophysectomized rats. In these animals the BE antiserum is still able to enhance the splenocyte proliferation. Our data suggest that BE exerts a tonic inhibitory effect on proliferation acting both centrally and peripherally, where immune-derived BE can inhibit proliferation throughout a paracrine/autoimmune mechanism. Manfredi et al., J.Neuroimmunol. 44:43-46, 1993.

814.10

To determine whether chronic neurochemical alterations could influence antibody production, the responses of two substrains of F1 ablise mice (Epilepsy Prone (EP) and Epilepsy Resistant (ER)) to immunization with sheep red blood cells (SRBC) were examined. Significant differences in levels of SRBC-specific IgG were found between the two substrains. In addition, significant differences in splenic lymph node spleen weights between the two substrains were observed, in both non-immunized and in immunized mice at several time points after immunization. The EP substrain showed elevated splenic NE levels and suppressed IgG production compared to the ER substrain. Treatment of ER mice with the β-adrenergic agonist (β,AR) terbutaline on days 4, 5 and 6 after immunization resulted in a significant decrease in IgG production relative to saline treated controls. This suggests that elevated splenic NE acting through βAR is capable of mediating the suppression of the IgG response seen as the EP strain. Taken together with previous studies indicating that the effect of elevated NE at the induction of the immune response can have an enhancing effect on antibody production, these data suggest a dual role for NE in the immune response.

814.11

Neurobehavioral manifestations in both human and murine models of systemic lupus erythematosus (SLE) have been correlated to diverse brain-reactive autoantibodies (BRAA). It is suggested that subsets of BRAA, especially IgG, bind to neuronal surface components and alter specific functions which may lead to some of these deficits. Using a natural murine model of SLE, we examined whether monocular IgG. BRAA and anti-neuronal IgG autoantibodies from sera are capable of altering voltage-gated sodium (Na+) and potassium (K+) channel currents of murine neuroblastoma NBP cells. Monoclonal antibodies (MAb) La22, that classically inhibits K+ currents produced from a B858 mouse/human hybridoma were used. NBP cells were incubated with the monoclonal BgA or anti-neuronal IgG exist which have specific functional effects on neuronal cells. These are hypothesized to be significant in the pathogenesis of immune-mediated neurobehavioral involvement in immunologic disorders.

814.12
THE EFFECTS OF SIX AND ESTRUS PHASE ON SURGERY-INDUCED INCREASES IN TUMOR CELL RETENTION AND ITS ATTENUATION BY MORPHINE. G.G. Pace*, S.A. Buon and S. Ben-Ezra. Ohio State Univ. College of Nursing, Columbus, OH 43210.

The vast majority of studies investigating the effects of surgery on immunity and tumor metastasis have used male subjects. We recently reported that morphine attenuates the metastatic-enhancing effects of surgery in male mice. The present study was designed to test the differing hormonal environments on female and male responses. Monoclonal findings indicating that females in proestrus/estrus (P/E, high estradiol levels) are more susceptible to tumor metastasis than are females in diestrus (D); those studies explored whether there were sex- or estrus-related differences in the metastatic-enhancing effects of surgery and in the effectiveness of morphine in attenuating this outcome. A summary of neurochemical data base, MAB106, suggests that the F344 rat was used. MAB106 cells metastasize only in the latter, a process known to be controlled by natural killer cells. In Exp I, females in P/E or D and males were randomly assigned to the surgery (standard abdominal surgery under halothane anesthesia), anesthesia only, or control group. In Exp 2, P/E or D females and males were evenly assigned to abdominal surgery with anesthesia or anesthesia alone, and were either treated or not with morphine (pre- and postoperatively in saline or a slow release suspension, respectively). Radiolabeled MAB106 cells were injected 3-1 h after surgery and marginals were removed 13 h later to assay their radioactive content. Surgery resulted in a 3-fold increase in tumor cell retention in all 3 groups, which was additive to the above-mentioned differences observed in the PE or DI females to both the anesthesia only and control groups. This finding may have important implications in the timing of surgical interventions for breast cancer. Morphine administration similarly benefited the males and females, evidenced by a significant interaction between the effects of surgery and morphine. Supported by the Oncology Nursing Society and NIH grant NS33829.
184.13


Using video imaging and vital fluorescent probes to visualize mast cell’s activation (NATURE 313:324, 1994) we have recently reported that electrical stimulation of splanchic nerve activated mesenteric mast cells (MeMCs) (Neurosci Abst 20:052, 1994) and that capsaicin-sensitive splanchic afferents is important for mast cell activation (Gastroenterology 1995, in press). Topical application of low dose capsaicin has been used to selectively excite the sensory terminals of thin primary afferents (Pharmacol Rev 43:147-148, 1991). Aims: to further determine the effect of capsaicin i.p. administration on the MeMCs and peritoneal mast cells (PMCs). Stalbofarmide B (200 μg/kg with capsaicin 5 x 10^(-3) M, 20 ml) was i.p. injected into urethane anesthetized rats and kept in situ for 20 min. Laporotomy was performed, pieces of mesentery were mounted on teflon tambours for MeMC examination and peritoneal lavage was harvested for PMC images. Images of MeMCs (n=18 to 87, median 45.5 cells) were randomly captured from each tambour, and PMCs (n=50 to 138, median 72 cells) from dish. For each experiment the stained cells of two tambours and two dishes were counted, normalized and expressed as mean ± SD. Data from three vehicle experiments were compared to these experimental trials. Results: capsaicin i.p. activated 76 ± 5.7% MeMCs as compared to 10.1 ± 4.3% N=5 vehicle trials (p<0.001 two-tailed paired t-test), whereas for PMCs the activated cell number was 9.7 ± 2.2% vs 10.6 ± 4.8% N=6 (p>0.7 two-tailed paired t-test). The results indicated that capsaicin (5 x 10^(-3)) i.p. activated MeMCs but not PMCs, suggesting the important of neural element involvement in the effect. Neutrophils staining to MeMC activation are further investigated. (Supported by NIH Grant NS 28433)

184.14

EXPRESSION OF NITRIC OXIDE SYTHASE TYPE II IN THE SPINAL CORD UNDER CONDITIONS PRODUCING THERMAL HYPERALGESIA. D. Graybiel* A. I. Koch, K. D. Gebhart and S. Murphy. Deps. of Pharmacology and Pathology*, Univ. of Iowa, Iowa City, IA 52242.

Peripheral tissue injury or nerve damage result in a thermal hyperalgesia believed to be mediated via the production of nitric oxide (NO). Attention is on NO derived from Type II NO synthase (NOS) but sustained production of NO via the NOS II isoform may be involved in the mediation of a transient thermal hyperalgesia produced in rats after intrathecal administration of proinflammatory cytokines. Male Sprague Dawley rats with an intrathecal sterile catheter were infused with IL-1β (10 ng) and IFN-γ (1000 U) and then sacrificed at various times. Segments of spinal cord (1 cm) centered around the lumbar enlargement were excised. Expression of NOS II mRNA was detected by RT-PCR and confirmed by ribonuclease protection assay. Western blotting revealed NOS II protein at 4 and 8 hours after cytokine injection. Expression was scattered diffusely throughout gray and white matter in cells which morphologically represent perivascular macrophages and/or vascular cells, glia, and small neurons. Immunohistochemical studies are in progress to identify these cells. There was no evidence of gliosis or infiltration by macrophages.

We conclude that endogenous cells in the CNS can be induced in vivo to express NOS II and that its expression in the spinal cord may play a role in transient thermal hyperalgesia. Supported by NS29226.

184.15


Interleukin-1β (IL-1β) is localized in the hypothalamus and responds positively after endotoxic administration. IL-1β has positive neuronal terminal fields included those in the arcuate nucleus. Since the arcuate nucleus contains a large population of β-endorphin (β-EP) neurons, we determined the effect of IL-1β in vivo and in vitro release of β-EP from these neurons. Furthermore, the role of nitrous oxide synthase in the IL-1β-regulated β-EP secretion is determined, because nitrous oxide, which is produced by this enzyme, has been shown to mediate IL-1β actions on various hypothalamic neuropeptides. In vitro release of β-EP was studied using primary cultures of rat fetal hypothalamic cells. IL-1β concentration-dependently decreased the release of β-EP from the cultured neurons. The inhibitory effect of IL-1β was completely prevented by a nitrous oxide synthase blocker L-NAMe. The blocker alone caused an increase in basal release of β-EP from the cultured hypothalamic neurons. In agreement with these findings, IL-1β and L-NAMe significantly altered hypothalamic β-EP release into the blood of intraperitoneally injected rats. IL-1β inhibited while L-NAMe increased β-EP levels in the blood of portal pituitary vessels. L-NAMe also prevented the inhibitory effect of IL-1β on portal pituitary blood levels of β-EP. These data suggest that IL-1β and nitrous oxide may interact to regulate β-EP secretion from the hypothalamus. Supported by the National Institutes of Health Grant AA08737.

184.16


The effects of cyclic nucleotides on the expression of the inducible nitric oxide synthase (iNOS) were investigated using an immortal astrocyte cell line (D30) derived from the mouse cerebral cortex. The expression of iNOS activity from these cells was dependent upon co-stimulation with interleukin-1β (IL-1β) and interferon-γ (IFN-γ), and was not induced by either cytokine alone. The induction of iNOS mRNA over basal levels was not apparent until 24 h and remained elevated out to at least 72 h. Accumulation of nitric oxide from the media stimulated iNOS activity was significant versus control cultures by 48 h and continued to increase out to the latest time tested (72 h). Co-stimulation with 8-bromo-cyclic AMP (100 mM) alone or in combination with IL-1β and IFN-γ did not have any affect on the levels of iNOS activity. Similarly, the addition of 8-bromo cyclic AMP (8-Br-cAMP), alone or in the presence of IL-1β or IFN-γ, did not result in detectable increases of iNOS activity. In the presence of both IL-1β and IFN-γ, however, 8-Br-cAMP increased iNOS activity and mRNA levels. The potentiation of iNOS activity observed from co-stimulation with IL-1β and IFN-γ, either in the absence or presence of 8-Br-cAMP, was inhibited by preincubating the cells with L-Nω-(i)-arginine (L-Nω-i-Arg), an irreversible iNOS inhibitor. These results indicate that iNOS-mediated processes can synergize with cytokines to stimulate iNOS expression in astrocytes. Moreover, since the requirement for either IL-1β and IFN-γ was not replaced by 8-Br-cAMP, additional signal transduction pathways are likely involved in the IL-1β and IFN-γ induced iNOS expression.
815.1 STATIC FORCE REPRESENTATION BY THE POPULATIONS OF MOTOR CORTEXULAR NEURONS IN THE JOINT-RELATED COORDINATE SYSTEMS. S. Tanaka*. Dep. of Electrical Eng., Sophia Univ., Tokyo 102, Japan

Dynamics representation in the motor cortex has long been suggested. Experiments by Kalaska et al. (J. Neurosci. 1989) provided deeper insights into the relations of the activities of motor cortical neurons to dynamics parameters. The objective of the present research is to investigate how force representation in the motor cortex. In order to do it, the descending circuit from the motor cortex to the spinal cord is modelled, which relates the population activity of M1 pyramidal cells to that of motor neurons. The activities of the cortical neurons are modulated sinusoidally by the direction of static force represented in the intraspinal, joint-related coordinate system (Tanaka, N.S.L., 1994). The main consequences of the theoretical model are: (a) The populations of M1 cells code static force of the arm; (b) the direction of the force is coded distributedly throughout the whole population of the cells; and (c) the magnitude of static force is coded by the depth of modulation of the activities of the M1 pyramidal cells; (d) the descending circuit transforms the static force coded in the motor cortex onto static joint torques, which are encoded by paired mononeuron pools in the spinal cord; (e) Change of the static joint torques with the postural change of the arm is provided by changing the activities of the pyramidal cells, rather than by changing the magnitudes of the neuronal firing; (f) A substantial proportion (but not more than half) of the pyramidal cell's negative correlation with the static force, which was observed in experiments (for example Maier et al., 1993).

815.3 CELL ACTIVITY IN MONKEY DORSAL PREMOTOR (PMd) AND PARARIetal AREA 5 CORTEX ARE ALTERED BY CHANGES IN ARM POSTURE FOR MOVEMENTS WITH SIMILAR HAND TRAJECTORIES. S.H. Scott, L.E. Seeger* & J.F. Kalaska, CNRI, Dept. of Physiologie, Univ. de Montréal, Montréal, PQ, CANADA H3C 3J7

We have shown previously that neuronal activity in monkey primary motor cortex (M1) is altered by changes in arm posture for movements with similar hand trajectories (Scott & Kalaska, Neurosci. Abstr. 20:982, 1994). The present study tested whether this modulation occurs on cells in the adjacent parietal area 5 (A5) and dorsal premotor (PMd) cortex. We trained two monkeys to move a pendulum-like handle to visual targets using two different arm postures: the 'natural' posture in the sagittal plane and the abducted approximate horizontal plane in order to grasp and move the handle. The two arm postures changed the mechanical state (i.e., length and EMG activity) of muscles that span the shoulder and elbow joints. In both postures, the elbow position remained at shoulder height. In both cortical areas, the activity of individual cells was usually not identical in the two postures. Tonic cell activity often significantly increased or decreased (PMd: A5: 45:50; M1: 30:53; p<0.05). The direction tuning of cells also often changed (PMd: 117:179; A5: 65:80; M1: 45:50; p<0.05) and the mean change in direction was less in PMd (22.6°) than in A5 (42.1°) and M1 (42.5°). Overall, the effect of arm posture was less for cells in PMd than in A5, and both these areas were less affected when compared to M1 or to mathematical models based on intrinsic movement attributes. Supported by MRC Group Grant in Neurological Sciences (FJK), and MRC (SHS) and FCAR (LES) Post-Doctoral Fellowships.

815.5 INPUT-OUTPUT PROPERTIES AND CHANGES OF RECRUITMENT GAIN OF THE CORTICOspinal PATHWAY. H. Deacme, B. A. Lassor, and C. Capella*. Centre de Recherche en Neurobiologie, Université Laval, Quebec, (QC), Canada, G1J 1Z4

Experiments were done to determine the form of the input-output relation of the corticospinal to the motoneuron pools of the first dorsal interneuronal (PD1) and the bitalial anterior (TA), respectively. The motor cortex was excited by focal transcranial magnetic stimuli (TMS). In both muscles the form of the input-output relation (i.e., recruitment recruitment gain) was sigmoidal. The steepness of the relation increased whereas the threshold decreased with increasing tonic background activation up to about 30-40% of the maximum tonic effort. The plateau level changed slightly by the background level, except that it could not be reached when no background activity was present. This was probably due to the limitation of the stimulator maximum output. However, this finding may reflect a change of the intrinsic excitability of the muscle fibres going from rest to activity, or that convergent inputs from different descending systems are required for maximal activation of motoneuron pools. The sigmoidal input-output relation implies that presentation of a test EMR is a function of the test EMR amplitude and that in fact there is no range over which the amount of facilitation or inhibition is either constant, or relatively so, as has been recently suggested for monosynaptic spinal reflexes (see Coenen et al., Exp. Brain Res., 81:35-45, 1990). The increase of the steepness of the relation with the background level of activity demonstrates that the recruitment gain (Cornell and Holmdal, Brain Res., 358:179-179, 1990) of the motoneuronal pools changes as a function of the recruitment level even for the same qualitative task. This implies that adjustment of the stimulus intensity to compensate for the effect of changes of the background level on the EMR amplitude is not a valid procedure to insure that the amount of facilitation or inhibition be independent of the test EMR amplitude.

815.6 MODULATION OF NEURAL ACTIVITY IN THE PRIMARY MOTOR CORTEX DURING DISCRIMINATION IN THE AWAKE MONKEY. W. Jiang and C. E. Chapman. CRNI, Université de Montréal, Canada.

Previous studies have shown that it is possible for monkeys to encode surface texture and it was suggested that such sensory information is important in the control of prehension grip under different conditions. The present study investigated the sensory properties in a monkey trained to discriminate a texture change in the surface of textured surfaces. The texture changes were applied to the contralateral fingerpad. The monkey was trained to discriminate a repetitive texture change in the surface (raised dots, 2 mm spatial period (SP) over the entire length) from 3 other surfaces in which the SP was proportionally increased to 3, 4 or 5 mm or the second half (4 mm) of the repetitive physically continuous surface. In order to discriminate each presentation of each surface, the monkey indicated the presence or absence of the surface and its texture by pressing a lever with the opposite hand to obtain a reward. Overall the monkey correctly discriminated between the different textures with both hands and had a receptive field (RF) that included the scanning digit tips (14 touch, 8 pressure, 9 joint) were vigorously active during discrete movement involving the scanning digits yet had no RF (n=10). Overall, 37 of the 41 units showed increased discharge to the presentation of the textured surfaces (4 cells not testing stimulation). Texture change-related discharge was seen in only 3/37 neurons in the task, and all 3 had a cutaneous RF that was tested for texture-sensitivity outside the context of the task (monkey not working; distracted with random drops of juice), 2/6 units (both had a cutaneous RF) now displayed texture-related discharge. The results indicate that texture-related information is indeed relayed to area 4, but texture sensitivity can vary as a function of the context within which the surfaces are presented. Supported by the MRC and Université de Montréal.
815.7
IMPORANCE OF STIMULUS INTENSITY ON THE TIME COURSE AND MAGNITUDE OF MOVEMENT-RELATED SUPPRESSION OF TACTILE DETECTION: HUMAN AND ANIMAL STUDIES (N. Curtain S.R. Image, E.J. Grant, E. Gruber, J. Granier, C. Joud, C. Nacher, S. Baucier, MCN, University of Montréal, Montreal, Québec, Canada, H3C 3J7)

Much debate surrounds the relative merits of active and passive touch. Previous studies have shown that active movement is accompanied by a reduction in the transmission of tactile cutaneous inputs. In order to evaluate the effect of a simple active movement on tactile detection, we used low intensity cutaneous stimuli; perceptual performance was examined in humans during the execution of a motor task, abduction of the right index finger. Stimuli were delivered to the glabrous tip of the right index finger. The subjective onset of stimulation was initially set at a level where 90% of the stimuli were perceived at rest (Po = 32 subjects). Four other stimulation intensities (1.25P0, 1.5P0, 1.75P0 and 2P0) were used. The separate blocks of trials consisted of the subject data. From individual subjects were pooled and the time course and amplitude of any movement-related suppression of tactile perception was examined in relation to two peripheral events: the onset of movement and the onset of movement-related electromyographic (EMG) activity. The time at which the first significant decrease in perception occurred increased from 210 ms before movement onset (190 ms before EMG onset) with stimuli of intensity Po to 10 ms before movement onset (30 ms after EMG onset) at intensity 2P0. The minimum proportion of stimuli detected during movement trials varied from 0% (complete abolition of stimulus detection) at intensity Po and 17% at intensity 2P0. These results indicate that movement-related suppression of tactile detection is highly dependent on the intensity of the stimulus being detected. Movement strategies in active touch could be designed to take advantage of this effect by optimizing the signal to noise ratio in conditions where there is a widespread increase in absolute detection threshold.

Supported by the MRC, FRSQ, GRSC and Unimédica.

815.9

Findings from studies using recording from single neurons in awake animals, have impelled us to investigate specific functional aspects of motor cortex. Our aim is to study the participation of motor cortex in different aspects of a voluntary movement. With this purpose, unitary activity has been recorded in motor cortex of cats previously trained in an operant conditioning. The process of conditioning consists in the presentation of an auditory stimulus (free field) with a random variable range of intensity (40dB, 70dB, or 100dB SPL). Three seconds later, the animal has access to the food by opening automatically a trap door. To eat, the cat must perform an extension movement of the forelimb followed by a flexion to get the food. Following this experimental protocol we studied the cellular discharge before and after the onset of movement of forelimb. Our results show that from twenty-eight cells tested, fifteen responded when the cat has the possibility to get the food. One-tenth had the greatest majority of cells in 250 ms. Thirteen cells of them, keep their increased simple spike discharge during 1-2 s. Perhaps, the eyes of the opening after the trap. The results suggest two kinds of populations cells in motor cortex, one related with voluntary movements and other with aspects related with the anticipation actions. (Supported by DGGCYT Research Project PB 91-0421).

815.11
SINGLE NEURON ACTIVITIES DURING A SENSORMOTOR DECISION J. Zhang1, A. Riehle2, S. Kornhuber1 and J. Requin*, 1Department of Psychology, University of Michigan, Ann Arbor and 2Cognitive Neuroscience Lab, CNRS, Marseille, France

A monkey was trained to perform wrist extension/flexion movements to align a pointer with a visual target while single unit activities in primary motor cortex (MI) were being recorded. The stimuli consisted of colored LEDs presented either to the left or right of a central starting position. Depending on the color of the LED, the monkey had to point either directly at it (compatible condition), or at LED on the opposite side (incompatible condition). Compatible and incompatible trials were blocked during training sessions, and were randomized within blocks during recording sessions. Neuronal activities were observed in two types of neuronal groups. The first type (compatible/incompatible) was analyzed to determine whether such activity was more related to stimulus side, response side, or the compatibility rule that maps one or the other. A LED vector was introduced to orthogonally decompose the pattern of neuronal activity (across four trial types) into the sensory, motor, and rule aspects of the task. The direction of the vector is represented by a rotation, which indicates the functional role of the activity (whether related to stimulus, to response, to rule, or to the monkey's "decision"), while the length of the vector reflects the differential activity change related with each neuron, the peak differential activity during a trial was identified along with its spherical (functional) locus. We find that the peak spherical locus of the recorded population (156 units) in MI are clustered and evolve from sensory -> "declosion" -> motor "landmarks" as a trial progresses, reflecting the monkey's sensorimotor transition during a trial of the task.

815.12
CONDITIONING OF MONKEY MOTORCORRELANT UNIT BIDIRECTIONALITY BY COMPLEXITY OF TRAINING PROGRAM. D. Sahrman*, M. Clare, T. Anderson, M. Toriguchi, C. Montgomery, Washington University School of Medicine, Department of Psychology, St. Louis, MO 63110.

Motor cortical neurone discharge patterns have been related to movement elements such as force and direction. The relationship of these neuronal patterns with a complex training program, that the MI neurons' discharge patterns has not been studied. In this study, units in animals performing a compound task involving continuous and reversal angle forces were compared with units in animals performing a simpler set of fixed force and direction tasks. Four different units were trained to perform an angular movement tasks in response to visual stimuli indicating magnitude and direction of force. Two animals were trained to perform a complex task involving fixed angle forces and small forces and reversals of force. Two monkeys were trained to perform a complex task involving fixed large forces. Standard extracellular recording techniques were used to record from hindlimb motor cortical area.

Related cells (667) had a significant increase in firing rate (p<0.05) with the distribution of trials from rest to dorsolateral (0) and to plantar (P). The direction of the cell was assessed from the distribution of cell types classified as unidirectional (UD), bidirectional (B) and multidirectional (MD). Cells trained with the compound task had a greater proportion of BI units than those trained with simple tasks. The BI/UD ratio for complex training were 148/107 (1.46) and for simple training 91/101 (0.99). The proportion of BI units for the small force task (MI, 52; BI: 80; ratio = 1.64) was similar to that of the large force task in the complex trained monkeys.
816.2 CODING OF TARGET MOTION AND HAND MOVEMENT PARAMETERS IN MOTOR CORTEX DURING TARGET INTERCEPTION. W. Kow* N. Lindman Post, A.P. Georgopoulos, A.B. Schwartz, J.A. Ashe, J.T. Thomas, Emory Univ., VAMC, and Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55417

We recorded the activity of 380 cells in the arm area of the motor cortex while a monkey intercepted a moving target on a computer screen using a 2D articulating manipulandum. The target accelerated, decelerated or traveled at a constant velocity of 0.5, 1.0 or 1.5 s. A multiple linear regression was used to relate the ongoing cell activity to the evoked position, velocity and acceleration of the target and hand movement. Since the interception movement was always upwards, the trial-by-trial-time course of cell discharge at time t was expressed as a function of the y-component of position, velocity and acceleration of the target, at time t+k, and the hand, at time t+l, where k and l were independent time shifts (−120 to +140 ms). The R^2 was calculated for each 10 ms shift. We found that in 378/380 cells the regression coefficient was statistically significant at the combination of shifts with the highest R^2 (mean R^2 = 0.149). In practically all cells both target and hand effects were significant; in only 1/378 cells there was only a significant target but no hand effect. These results indicate that the motor cortex processes dynamically time-varying information concerning both target and hand movement. The same analysis was performed between the average (across cells) target and hand movement parameters and the y-component of the time-varying neuronal population vector. The highest R^2 (0.951) was obtained at t = −90 ms and l = +100 ms. The regression coefficients for position and velocity of target and hand movement were statistically significant (p < 0.05) but not those for target or hand acceleration. These results indicate that the moving target exerts an ongoing, dynamic influence on population activity with a latency of 90 ms, and that, in the neuronal population exerts an influence on the hand movement with a latency of 100 ms.

816.4 SET-RELATED ACTIVITY IN THE DORSAL PREMOTOR AREA REFLECTS TARGET LOCATION RATHER THAN LIMB TRAJECTORY. L. Shen* and G.R. Alexander, Dept. of Neurology, Emory Univ, Sch. Med., Atlanta, GA 30322

The dorsal premotor area has been implicated in the preparation for movement, but the precise contribution of this region to motor control processes remains unclear. Single neuron activity was sampled from the dorsal premotor area and from primary motor cortex in two macaque monkeys while the subjects performed a visually instructed, delayed reaching task. Subjects used the right forearm to move a joystick, whose movement was recorded by a camera presented in a video display in front of the animal. Direct vision of the limb was prevented by an occluding collar. Each trial began with the illumination of a fixation point in the center of the display. After the subject aligned the cursor with the fixation point, four radially arranged peripheral targets were also illuminated. After a variable delay, the subject was instructed, by the brief dimming of the appropriate peripheral target, which of the four would be the "correct" target for that trial. A second variable delay then ensued until the fixation point dimmed, which served as the movement-triggering stimulus. At this point, the subject was required to align the cursor with the correct peripheral target by moving the joystick in the appropriate direction. The direction of the target (relative to the fixation point) was dissociated from that of the hand/limb trajectory by varying the spatial mapping between joystick and cursor. This made it possible to determine whether the set-related activity that preceded the movement to capture the peripheral target was preferentially related to the direction of the forthcoming hand/limb movement or to the spatial properties of the target. Of the dorsal premotor area neurons with directional, set-related activity, nearly all showed directional tuning that reflected the direction/location of the target rather than that of the hand's trajectory. In contrast, in primary motor cortex roughly one half of the neurons with directional, set-related activity showed directional tuning that reflected the hand's trajectory while the other half reflected the direction/location of the target.
1.7 COMPARISON OF THE NEURONAL ACTIVITY IN SMA AND IN THE VENTRAL CINGULATE CORTEX DURING PREHENSION IN THE MONKEY. S. B. Aizenman, M. A. Smith, C. R. S. N, University of Montreal, Quebec, Canada.

A total of 92 neurons in the cingulate cortex, and 115 neurons in SMA were recorded from both grasping and lifting in 2 M fasciculatus monkeys. Neurons in SMA were located in the medial wall, just caudal to the genu of the arcuate sulcus; neurons in the cingulate cortex were more rostral and ventral. The cingulate sulcus caudal to the hand representation of SMA, in area 23c. The number of cells with propulsive behavior was considerably higher than that of cells receiving cutaneous afferents in both the cingulate cortex (29.8%) and SMA (27.3%). Hand movements could be evoked by ICMS at 8 recording sites in the cingulate cortex and an additional 12 sites in SMA. In the samples of cells, the majority of the neurons increased their firing rate between 100 and 600 msec before the grip onset, with the same distribution of onset times. The proportion of phasic-tonic cells was lower in the cingulate cortex than in SMA. Forcing the monkey to perform the object-during the holding phase elicited excitatory responses in 19% of the SMA neurons and 32% of the cingulate neurons, at a mean latency of 49 msec in both groups of cells. No evidence of preparatory responses to the perturbation was found in the cingulate cortex, and only 5 cells in SMA exhibited a preparatory response. These results indicate that these 2 medial areas share some features and are involved in the sensorimotor control of the hand. However, both regions seem to be insensitive to the preparatory grip force increases in anticipation of the perturbation. Supported by NRC of Canada.

8.9 DIRECTION OF SACCADES EVOKED BY INTRACRANIAL MICROSTIMULATION (ICMS) OF SUPPLEMENTARY EYE FIELD (SMA) IS DEPENDENT ON BEHAVIORAL TASK CONDITION. B. R. McLaughlin, M. B. Riehle, and J. B. Strick. Department of Neurosurgery, University of Chicago, Chicago, IL, 60637.

In previous reports, the direction of saccades evoked by ICMS of the SEF has been characterized as goal directed, but at times as fixed vector. We report here that properties of saccades differ greatly depending on what oculomotor task the animal performed, specifically when the target was presented in a plane with five LEDs, which the monkey performed a visually triggered saccade task with delay. When the animal placed his hand on a hold plate, an LED was turned on as a fixation target, if fixation was maintained for 500 ms (no fixation), and the LED was damped as a GO signal. An LED, serving as a target, appeared approximately 500 ms before the GO signal, hence there was a delay between the appearance of target and the GO signal. ICMS was delivered to SEF at two different phases of the behavioral task. 1) During fixation, 500 ms after the initiation of the fixation, 2) immediately (50-100 ms) before the GO signal (pre-GO signal).

During fixation, the direction of saccades evoked by ICMS was always contrateral to cortical stimulus sites, e.g., (from left SEF, the evoked saccades were directed rightward. The saccade trajectory appeared either goal directed or constant vector, with a mean latency of 124.7 ms. In contrast, when the pre-GO ICMS was applied, the direction of evoked saccades altered depending on the position of the preceded target. Thus, the animal captured the target with a saccade, despite the presence of the ICMS. If the saccade target was presented contralateral to the stimulus site, the interval between the GO signal and the saccade onset was <100 ms. If the target appeared ipsilaterally, the interval ranged from 150-200 ms.

8.10.1 OSCILLATIONS IN MONKEY MOTOR CORTEX DURING VISUALLY GUIDED REACHING MOVEMENTS. B. Azuma, C. Ogata, H. N. Sato, and J. P. Donoghue. Department of Neuroscience, Brown University, Providence, RI 02912.

We previously described two rhythmic (25-30 Hz) local field potential oscillations (LFPs) in monkey primary motor cortex (ML, Sato and Donoghue, PNAS 90:4470, 1993). The y-LFPs occurred throughout a pre-movement delay period, ceasing around the saccade. They also occurred when the monkey changed behavior from quiet sitting to task engagement, suggesting that they are related to motor preparation. We now report that the y-LFPs enhance movement direction prediction. We record y-LFPs in ML through chronically implanted microelectrodes in a movement visually guided reaching task. 12 (3 targets x 4 animals) monkeys were trained for a non-motor task. At first, the monkey position the hand at a central point for an initial hold period in each of two tasks. In the no-movement condition, an illuminated fixation point for 800 ms. After a 4 s "pre-cue" period, all targets were displayed, and the monkey was required to move a position feedback cursor to the instructed target within 600 ms after the go-cue. In the visual task, the target remained illuminated throughout the pre-cue period. In both conditions, distinct y-LFPs were evident during both the hold and pre-cue periods, but they diminished around movement onset and remained low throughout the movement. We conclude that y-LFPs are not related to preparation for a specific movement direction because they occurred during the hold period (before movement direction was specified), and they showed vector direction. Additionally, no apparent difference in the pattern or occurrence of y-LFPs was seen between the memory and visual tasks. Synchronized activity y-LFPs were sometimes evident across multiple sites, but these were not temporally locked to any task event. These finding indicate that oscillations are not related to activity of the memory processes that might occur within ML, but that they are inversely related to motor execution. Supported by Grant NS 25074.


Simultaneous recording of adjacent motor cortical neurons reveals rapid modifications in their synchronized activity during the execution of various motor tasks. These modifications are time-locked to the occurrence of other external events, such as the presentation of visual stimuli, or internal events, such as the time when anticipatory responses are triggered. In a monkey performing a saccade, directional saccade response is necessary to reach another fixation. Coincident firing enhances the probability that the receiving neuron will be activated. Hence, coincident firing might be considered as a potential neural code used to bind both discrete and continuous sensory events. The interactions of neurons that project to the vestibular nuclei are driven by the saccade task, which allows for the analysis of the firing rates of the neurons involved. The presence and significance of these events are considered together with the non-stationarity in the firing rates of the neurons involved. The results suggest that the degree of synchronization in groups of motor cortex neurons might play a role in the neural control of movement. These results have important implications for the understanding of the execution of a motor task. Furthermore, the structure of the interaction between neurons under the hypothesis that a single neuron might participate in different motor groups is described. Supported by Grant NS 3058/2006.
816.13

The possibility that temporally precise events participate in cortical information processing was tested. Multiple single units were recorded in the frontal cortex of Rhesus monkeys during performance of a delayed response task. We searched for firing patterns (FPs) that reliably repeated in trippers with specific time intervals (with jitter of ±1 ms). The estimation of significance took into account the firing rates and the pairwise correlation amongst the units.

We found that (1) FPs were composed of spikes of one or more units, spanning few to hundreds ms. A given triplet of units exhibited FPs, of different temporal structure. (2) Many FPs appeared in relation to behavior. Moreover, different FPs of the same units could appear repeatedly during different behavioral modes. (3) FPs tended to appear in clusters, each dominated by a different set of units. The clusters were also related to the behavior. (4) Cross-correlations (CC) were computed for spike trains and compared to the corresponding FPs triggered CCs. For about 60% of the neuronal pairs, the two CCs had different shapes. (5) The range of observed time intervals in FPs of a given neuronal pair, was either broad (presumably corresponding to a weak coupling) or narrow (presumably corresponding to a strong coupling). (6) Some FPs appeared more frequently in association with the occurrence of certain other FPs.

The findings support the hypothesis that information processing is mediated by synchronous activations of neuronal groups, allowing single neuron to participate in different computational processes.

816.14
NEURAL NETWORK MODELING OF MOTOR CORTICAL OPERATIONS DURING MENTAL ROTATION AND MEMORY SCANNING TASKS. A.V. Lakshmi, R.R. Amirkhan, V.J. Moteabar, and A.P. Georgopoulos, Brain Sciences Center, VA Medical Center, Minneapolis, MN 55417.

We propose a neural network model that reproduces quantitatively the spiking activity of motor cortical cells recorded in behaving monkeys during performances of two different visuomotor tasks: (i) the mental rotation task, which required the production of a movement at an angle from a stimulus direction, and (ii) the memory scanning task, which required the selection of an appropriate movement direction, depending on the serial position of stimuli in a sequence. The present study documents an ensemble of directionally tuned motor cortical cells is simulated by a recurrent network of interconnected, stochastic spiking neurons. The key point of the model is that a large repertoire of neural activity is permanently stored in the connectivity matrix in such a way that, once it is initiated, a particular neural dynamical evolves in time as a self-sustained dynamic attractor of the network. Our simulations demonstrate that specific patterns of motor cortical activity observed in experiments using paradigms (i) and (ii) can be stored in the connectivity matrix and can be initiated by specific external inputs of short duration.

816.15
HIGHLY NONLINEAR PROCESSING OF TEMPORAL INPUT FLUCTUATIONS BY REALISTICALLY MODELED CORTICAL BURSTING NEURONS. P.H. Bedenbender* and M.M. Mergner. Beck Center for Integrative Neuroscience, Univ. of California at San Francisco, San Francisco, CA.

Analytical models of spiking neurons, MacGregor (1987) model neuron, and sigmoid elements mix all the (and nonlinearly distinct temporally fluctuating inputs (Bodenbender, 1995). Inputs to these models must therefore be highly synchronous to produce synchronous outputs. All of these models have simple dynamics characterized by the kinetics of post-synaptic potentials. Through computer simulation, we studied whether or not the (intrinsic properties, and longer time constant spikes, as well as calcium exchange, diffusion and modulation) of a bursting neuron would represent temporally fluctuating inputs more accurately than do these simpler models. A one-compartment model of a cortical bursting neuron based on a model by Lytton and Sejnowski (1991) was simulated with the NEURON. Pairs of model neurons received input from partially overlapping pools of Poisson spike trains. Simulations were run with both low variance input (1000 presynaptic neurons) and high variance input (1000 presynaptic neurons). For both sets of simulations, the synaptic strength was varied to evolve firing rates ranging from less than 4 spikes per second to over 90 spikes per second. We measured the output correlation coefficient as a function of the fractional shared input. These quantities would be equal for a system that represents temporally fluctuating inputs well. In every case, the output correlation coefficient was far less than the fraction of shared input, just as for the more simplified models. We conclude that neurons that behave like this model neuron are better suited for responding unambiguously to particular synchronous spatial input patterns than is accurately representing fluctuating inputs with a fluctuating output spike train.

Special thanks to Michael Iles for help with NEURON. Thanks to Bill Lytton for the calcium pump/diffusion model. Supported by NIH NS3044 and Pittsburgh Supercomputing Center.

816.16
NONLINEAR DYNAMICS OF SPONTANEOUS NEOCORTICAL FIELD POTENTIALS RECORDS DURING ANESTHETIZED AND Awake STATES IN CHRONICALLY IMPLANTED Rats. Mark E. Jackson* and Larry J. Carlen, GR4117, Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Nonlinear dynamics provides a means of analyzing multi-variable, complex systems that were formerly considered either random or too complex to understand. The analysis of the correlation dimension of a one-dimensional time series can reveal the deterministic nature of the complex system and allows an estimation of the minimum number of variables necessary to describe that system. We are using nonlinear dynamics to analyze the cortical activity of rats with the goal of determining the essential neurophysiological constituents of functional cortical activity. This study describes an intact rat preparation with chronically implanted electrodes in SI, MI, and II neocortex. Spontaneous field potentials were recorded during deep nembutal anesthesia and during non-moving, alert activity. The autocorrelation function showed a slow fall-off and an extended range of positive correlation and the power spectrum showed broadly spaced peaks, typical of a fractal signal. Samples of the time series (32 sec at 1 msec sampling interval) were analyzed using the Grassberger-Proccacia method to determine the correlation dimension. The dimension in the awake state was higher than the anesthetized state (>2.9 vs. 1.2). We plan to use this preparation to study cholinergic modulation in the neocortex of awake, behaving rats. Supported by a grant from the Whitehall Foundation.

817.1
BLOCKADE OF HALOPERIDOL-INDUCED CATALYSIS BY 8-OH-DPAT IS MEDIATED BY FOREBRAIN RECEPTORS. K. Ebele-Wang, B.A. Balsa, and M.F. Chesseler, University of Pennsylvania, Department of Pharmacology, Philadelphia, PA 19104.

Peripheral administration of the prototypical dopamine receptor antagonist haloperidol, elicits catalysis in rats. Studies by Neel-Bellevue et al. (1993) demonstrated that systemic administration of the 5-HT(1A) agonist, 8-OH-DPAT, blocked haloperidol-induced catalepsy at the location of the 5-HT(1A) receptors mediating this response is unknown. In the present study, we have compared the effects of 8-OH-DPAT administration into the ventral tegmental area (to act on forebrain 5-HT(1A) receptors) vs. infusion into the cingulum bundle (to act on spinal cord 5-HT(1A) receptors) on haloperidol-induced catalepsy. Male Sprague-Dawley rats were implanted with chronic guide cannulae for intracerebroventricular (ICV) or intracranial (ICM) administration. All rats were tested once daily after haloperidol (2 mg/kg, sc.). Five days later, the animals received the same dose of haloperidol and catalepsy was recorded at 1, 2, 3, and 4 hours after injection. To test for catalepsy, rats were placed with forepaws resting on a horizontal bar 11 cm above the bench top and the latency to replace both forepaws on the bench top was measured (max test 300 sec). Fifteen min prior to the 3 hour observation, 8-OH-DPAT (10/25/50) or saline was administered ICV or ICM according to a within-subjects design. ICV administered 8-OH-DPAT significantly reduced catalepsy at 5/8/16 of the duration of the catalepsy at the 3 hour point, 15 min after its infusion. However, catalepsy returned to control levels by the fourth observation period, 75 min after 8-OH-DPAT infusion. ICM administration of 8-OH-DPAT had no effect of haloperidol catalepsy. These data confirm and extend previous studies demonstrating that 5-HT(1A) agonists block haloperidol-induced catalepsy in the rat. Furthermore, the data suggest that 5-HT(1A) receptor mechanisms in the forebrain, rather than in the spinal cord, are responsible for mediating the antiparkinsonian effects of 8-OH-DPAT. Supported by NIH grants MH49849 and MH48125 and Tourette Syndrome Association.

817.2
ΔFosB PARTICIPATES IN THE MEDIATION OF PRIMING G.S. Robertson* and M. Moseley* Dept. of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5. Dept. of Toxicology, University of Cagliari, Viale A Dazio 182, Caligari 09100, Italy.

Administration of dopamine receptor agonists to rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal system produces changes in the denervated striatum that enable a subsequent injection to elicit more vigorous circling. This behavioural phenomenon is termed the D1-like dopaminergic receptor agonist induced late-onset of spontaneous circling (SIC) in the denervated striatum. In order to determine whether ΔFosB is involved in priming, we examined whether (A) levodopa-induced priming and ΔFosB expression have similar time courses and (B) if inhibition of ΔFosB synthesis with an antisense oligonucleotide reduces levodopa-induced priming. ΔFosB expression in the 6-OHDA-denervated striatum peaked 3 days after levodopa administration and returned to preinjection levels at 10 days. This time course is similar to that for levodopa-induced priming. Moreover, intrastriatal injection of an antisense, but not a random, oligonucleotide against ΔFosB mRNA 14 hours prior to levodopa administration significantly reduced circling to a subsequent injection of the selective D1-like agonist SKF 38393. Taken together, these findings suggest that ΔFosB may play a role in those intracranial events which mediate levodopa-induced priming.

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817.3 THE PENDUCULOPONTINE Tegmental Nucleus as a Striatal Output Station. I. Effects of Microinjections of GABA Agonists and Antagonists on Stereotyped Behavior. L.F. Allen, M.P. Latimer, R.J.J. McConnell, A.S. Wallis and P. Winn. School of Psychology, Univ. St Andrews, Fife, Scotland KY16 9ST.

Evidence from the striopallidal or PPTg disinhibits orofacial stereotypies stimulated by 6-OHDA in the ventrolateral caudate-putamen (VLCP). Allen LF, and Winn P, Exp Brain Res 1995, in press.

Anatomical and behavioral evidence indicates that both dorsal and ventral projections are processed through the PPTg but it is not clear what particular role this structure fulfills. There are several VLCP output sites from which orofacial stereotypies can be generated by microinjecting GABAergic drugs. In the present experiments we have sought to first, confirm that microinjections of GABA drugs into the substantia nigra pars reticulata (SNr) elicit orofacial stereotypy and, second, examine the effects of various doses of the GABA agonist muscimol and the anticholinergic microinjected into the PPTg. In each case rats' performance was videotaped and scored using a checklist. In the first experiment, 750 ng/0.5 ml muscimol injected into anterior SNr stimulated licking and wetting, directed mainly at the cage walls. Injections into posterior SNr significantly less effect. 750 ng/0.5 ml picrotoxin had no effect on orofacial activity when injected into either anterior or posterior SNr. In the second experiment, 15, 30 and 450 ng/0.5 ml of either muscimol or picrotoxin microinjected into the compact portion of PPTg had no effect on orofacial stereotypy. Small changes in the incidence of sniffing and rearing were observed. These data suggest that while loss of the PPTg disinhibits orofacial stereotypy stimulated from the VLCP, manipulating GABA transmission in the PPTg in the absence of other stimulation does not induce significant changes in orofacial or other forms of unconditioned behavior.


Previous electrophysiological studies have suggested that the subthalamic nucleus (STN) is an important source of excitatory glutamatergic input into substantia nigra (SN). To evaluate the functional significance of these inputs in the awake, behaving rat, we compared the effects of focal STN pharmacological inhibition with the effects of blocking glutamate receptors within SN. Intermittent muscimol (200nmol), a GABA(A) agonist, or bicuculline methiodide (BIC) injection into SN did not induce stereotypic contraversive postural asymmetry without stimulation of locomotor activity.

A similar response was evoked by the direct infusion of kynurenic acid (1000nmol), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, into SN. These results contrast with the hypothesis that excitatory glutamatergic inputs into SN regulate postural control and that the STN is a source of these inputs. The alteration in locomotor behavior following inhibition of SN or blockade of glutamate transmission in SN was in clear contrast to the marked locomotor stimulation (in addition to postural asymmetry) induced in the same animals following direct unilateral application of muscimol in SN, an effect that has been previously well characterized. It therefore appears that these two STN-derived and STN-unrelated glutamatergic inputs have opposite effects on the modulation of locomotor activity.

Supported by NIH grants #NS05276 and F31 MH010812


The excitotoxic model of striatal damage has been used extensively in the rat as a test to motor deficits both in whole body asymmetries and in skilled limb movements. However, conflicting results of both the type and extent of behavioural deficits have been reported. Unilateral excitotoxic lesions of the dorsal striatum typically give rise to ipsilateral rotation in response to both amphetamine and apomorphine. Conversely, recent studies have claimed that a lesion in the retrolateral striatum induces a marked contralateral rotation in response to apomorphine. Bilateral excitotoxic lesions placed in the dorsolateral striatum cause a marked impairment in reaching movements of the forelimbs and tongue and lesions to more ventrolateral regions result in impulsive and stereotyped reaching movements.

The present study investigated the effect of unilateral biotin acid lesions in the dorsal striatum on rotation in response to both amphetamine and cocaine and in the “staircase task” of skilled forelimb use in a 2x2 matrix design experiment. Adult female Sprague-Dawley rats received a double unilateral lesion of 0.5 µl 0.06M biotin acid at 2 sites in either the anterior, posterior, medial or lateral striatum. Rats which received posterior lesions showed a marked ipsilateral rotation in response to both amphetamine and cocaine, while animals receiving anterior lesions showed a marked contralateral rotation in response to cocaine. Rats receiving lateral lesions showed a marked impairment of contralateral paw use on the “staircase test” while animals with medial lesions showed no significant difference to control operated animals. The motoric expression of the somatomotor organization of the dorsal striatum in its role in motor functions and indicate the necessity for design of lesion and transplantation studies to take into account the locus of an excitotoxic lesion in order to evaluate the behavioral deficits and recovery which result.

817.8 PREFERENTIAL LOCALIZATION OF SELF-STIMULATION SITES IN STIROSOMES/PATCHES OF RAT CAUDATE-PUTAMEN. N.M. Whelan and M. Eliot. Department of Psychology, McGill University, Montreal, Quebec, Canada.

The striatum consists of two histochromically distinguishable compartments: “patches” or “striosomes”, surrounded by a “matrix”. As a test of the hypothesis that the striosome/patch compartment may have reward-related functions, this study examined the role of the two striatal compartments in electrical self-stimulation of the brain. Rats were implanted with monopolar electrodes aimed at the medial portion of the caudate-putamen. After recovery from surgery they were trained to bar press for 0.5 sec trains of 60Hz sine-wave stimulation. Training consisted of placing the rats in the test cage and “shaping” them to press the bar by administering trains of stimulation contingent upon behaviors that approximated the bar pressing response. Following each training session each rat remained in the cage for 30 min to test for spontaneous bar pressing. All rats were naive until they met the self-stimulation criterion — at least 100 spontaneous responses in 30 min on 3 consecutive days or for a maximum of 11 - 15 days. After maintaining or failing to attain the criterion the animals' brains were stained for calbindin or calretinin. Animals with electrodes that were in or touching areas of light calbindin (Giesler, et al, Proc.Nat.Acad.Sci.U.S.A 82:8780-8784,1985) or heavy calretinin (Elias, in preparation) staining, indicating contact with a patch/striosome, acquired self-stimulation behavior more reliably than animals with electrodes terminating exclusively in tissue expressing heavy calbindin or light calretinin staining (matrix). The results provide in vivo evidence that the striosome/patch compartment is functionally differentiated from the matrix compartments. Direct stimulation of striosome/patch compartment, but not of the matrix, led to acquisition of bar pressing behavior, either because striosome stimulation has rewarding consequences, or because stimulation of this area promotes the storage of new information.
817.9 INTRASTRIAL DNQX INDUCES CONTRALATERAL ROTATION AND GLOBUS PALIDUS FOS IN DOPAMINE-DENERVATED RATS J.J. Schubert* and LF Marshall, Dept. of Psychology, University of California, Irvine, 92717. The striatopallidal and striatogiral projections are the two major output pathways of the striatum. These pathways both use GABA as a neurotransmitter, and are influenced by dopaminergic and serotoninergic afferents. Several measures of neuronal activity indicate that interruption of dopaminergic neurotransmission in the striatum leads to increased basal activity in striatopallidal neurons. We have examined the role of striatal glutamatergic neurotransmission by combining local glutamate antagonist application with behavioral quantification and immunocytochemical localization of Fos in the globus pallidus (GP) as an index of nonnal striatopallidal activity. Rats were given unilateral s-ODN lesions of the nigrostriatal pathway. 24 h post lesion, each rat was implanted unilaterally with a guide cannula aimed at the anterior portion of the denervated striatum. Unlesioned control rats were also implanted with striatal cannulas at this same 3-4 d following cannula implantation all rats were infused intracranially with the AMPA receptor antagonist DNQX (0.75, 1.5, or 3.0 µg) or vehicle (rat), and perfused 2 h later. The fixed brains were sectioned and processed for Fos immunoactivity. Rats with 6-ODNA lesions displayed dose-dependent contralateral rotation in response to DNQX, but not to its vehicle. Unlesioned control rats were unresponsive to either DNQX or its vehicle. DNQX dose-dependently induced Fos in the GP of 6-ODN-lesioned rats but not in unlesioned control rats. Vehicle did not induce significant GF P in either lesioned or unlesioned rats. Based on these data we conclude that glutamatergic input to striatal AMPA receptors contributes to DA-derivation-induced hyperactivity in the striatopallidal pathway. Furthermore, the disinhibition of pallidal neurons may play an important role in the expression of motor behavior.

817.11 ANTISENSE OLIGODEOXYNUCLEOTIDES: COMPARISON OF THE UPTAKE AND LOCALIZATION OF PARTIAL AND FULL PHOSPHOROTHIOATE DERIVATIVES IN BRAIN. H.A. Robertson*. Lab. Molecular Neurobiology, Dept. Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4H1. Antisense oligodeoxyribonucleotides (ODNs) represent a powerful new technique for manipulating gene expression in vivo. Endo- and exonucleases present in tissues rapidly degrade antisense ODNs thus limiting their effectiveness and in general, most people have worked with antisense ODNs where the phosphodiester bond is modified to a phosphorothioate bond. However, we and others have found fully phosphorothioate derivatized ODNs to be toxic when administered chronically. In an attempt to reduce toxicity, we have studied short 21 nucleotide ODNs with only one single phosphorothioate linkage at the 5' and 3' ends of the ODN modified to phosphorothioate derivatives (chimeric ODNs). Behaviourally, we observed that the onset of activity for such chimeric antisense ODNs directed against a gene was much quicker than that for the fully-phosphorothioated ODNs. This has been examined using FITC labelled antisense ODNs. Rats were injected directly into the striatum with 2 µl of either a chimeric antisense ODN to c-fos or a fully phosphorothioated antisense ODN to c-fos. At various times up to 24 h after injection, the animals were perfused, sections cut and the uptake and localization of the ODN was followed using fluorescence microscopy. The chimeric antisense ODN to c-fos appeared to diffuse more rapidly in striatum and was completely gone at 24 h; the fully derivatized ODN was still present at 24 h. Interestingly, both the chimeric and the fully phosphorothioated ODNs appear to be selectively taken up by neurons in globus pallidus. (supported by the MRC of Canada and SmithKline Beecham Pharma (inc).

817.13 CONTROL OF RESPONSE FORCE IN RATS: EFFECT OF BILATERAL PARTIAL 6-OHDA NIGRAL LESIONS AND DOPAMINERGIC DRUGS. J. Berman*, R.E. Strecker and X. Liu, Dept. of Psychology and Psychiatry, SUNY, Stony Brook, NY 11794-2500. The cardinal signs of Parkinson's disease (PD) include bradykinesia and tremor. In addition, PD patients show deficits in isometric force production and the tremor is considered an individual movement. We have dissected the mechanism of these behavioral deficits we examined changes in both the force and timing of individual isometric responses in a rat model of PD (Liu et al., this volume). Hungry rats were trained to apply force to a central beam for 2 s. Following beam release, visual stimuli were presented to signal which of two other beams (left or right) should be pressed. A sugar water reward was made available if the left beam was pressed with low force (<3 g), or if the right beam was pressed with high force (>50 g). Over the course of training rats made approximately 80 long-duration responses, 100 high-force responses and 100 low-force responses. Bilateral, partial 6-OHDA nigral lesions resulted in significant increases in the number of low-force errors. These errors were entirely due to loss of control over the rate of rise of force (dFd). Response timing was unaffected either during responses that demanded force control or during responses that required duration control. These results are attributed to a deficiency in the programming of response force rather than to deficiencies in the on-line regulation of motor output (see Slikhst & Breen, this volume). Related results from pre-lesion drug probes with amphetamine, haloperidol and amantadine will also be reported.

817.10 TIME- AND DOSE-DEPENDENT EFFECTS OF THIO END-CAPPED ANTISENSE OLIGONUCLEOTIDES ON AMPHETAMINE-INDUCED STRIATAL c-FOS EXPRESSION AND BEHAVIOUR IN THE RAT. L. Hunte*, B.J. Chisham, K.M. Murphy and H.A. Robertson, Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H1. Previous work demonstrated that intracerebroventricular (i.c.v.) infusion of a fully substituted anti-sense oligonucleotides (s-ODNs) targeted to c-fos mRNA attenuates striatal c-fos expression and results in isopivperine rotational behaviour in rats challenged with amphetamine, however, multiple infusions of this s-ODNs into the brain were found to produce neurotoxic damage. Although a reduction in the number of these substitutions may minimize neurotoxicity, few studies have examined the effect of partial substituted anti-sense ODNs in vivo. In the present study, the effects of single and double end-capped s-ODNs targeted to c-fos mRNA on apomorphine-induced striatal c-fos expression and rotational behaviour were examined in rats. Intrastriatal infusions of a single end-capped s-ODN (2 nmol) 1, 2.5 or 5 µg but not 10 hours prior to amphetamine challenge (5 mg/kg i.p.) attenuated striatal c-fos expression and resulted in isopivperine rotational behaviour in a time-dependent manner. Similarly, infusions of a single or double end-capped s-ODN (0.5- 6 µmol) 2.5 hours prior to amphetamine challenge resulted in similar effects in a dose-dependent manner. However, at the higher doses of these antisense s-ODNs (4-6 µmol) some animals demonstrated seizure activity and increased c-fos expression was observed in the cortex, thalamus and lateral striatum in addition to an aspical pattern of amphetamine-induced c-fos expression in the contralateral striatum. Thus, the present data suggest that infusion of low dose single or double end-capped s-ODN is effective in attenuating striatal c-fos expression and inducing isopivperine rotational behaviour in amphetamine challenged rats while at higher doses both s-ODNs lead to seizure activity in some animals. [Supported by The Parkinson Foundation and Medical Research Council of Canada]

817.12 INDICES OF ON-LINE AND PREPROGRAMMED CONTROL OF OPERANT RESPONSE FORCE IN RATS. A.B. Slikhst* and J. Breen, Dept. of Psychology, SUNY, Stony Brook, NY 11794-2500. Research on human subjects has shown that control of Peak response force (PF) by Time to Peak Force (TPF) reflects the operation of feedback (FB) processes, whereas control of PF by the Rate of Rise of Force (dFd) reflects the operation of feedforward (FF) processes. The current study tested whether TPF and dFd have the same relationships to FB and FF control of response force in rats. Knowledge-of-Results (KR) (Immediate or Terminal) and Force Contingency (LOW [2 g] or HIGH [18 g]) were examined in a 2 x 2 between-subjects design. Under conditions of Immediate KR, food reward delivery occurred at the moment during response execution that the imposed force requirement was achieved. This afforded the opportunity for response force to be regulated by the available external force. It was anticipated that this would make use of the Immediate KR in controlling PF, whereas HIGH-Low differences in PF would be determined by HIGH-Low differences in TPF. Under Terminal KR, use of information about response correctness during response execution was prevented since food for responses meeting the imposed force requirement was delivered at response end. Under these conditions, rats were forced to rely on FF control and it was anticipated that HIGH-Low differences in PF will be determined by HIGH-Low differences in dFd. Both expectations were confirmed indicating that in rats, as in other species, modulation of PF by TPF reflects FB control whereas modulation of PF by dFd reflects FF control.

817.14 DEFICITS IN RESPONSE SELECTION PRODUCED BY BILATERAL PARTIAL 6-OHDA NIGRAL LESIONS AND DOPAMINERGIC DRUGS. X. Liu*, R.E. Strecker and J. Breen, Dept. of Psychology and Psychiatry, SUNY, Stony Brook, NY 11794-2500. A sensitive behavioral test was developed to track changes in sensorimotor capacity produced by partial nigral lesions in rats. Hungry rats were trained to apply force to a central beam for 2 s. Following beam release, visual stimuli were presented to signal which of two other beams (left or right) should be pressed. A sugar water reward was made available if the left beam was pressed with low force (<3 g), or if the right beam was pressed with high force (>50 g). Following collection of the reward, stimuli instructed the animal either to repeat the last response or to start a new sequence by pressing the central beam. The task was designed to measure simple and choice reaction times as well as to record various sorts of response errors. After bilateral 6-OHDA nigral lesions, which resulted in a loss of DA neurons in substantia nigra and 50-80% forebrain DA depletions, RT lengthened as an inverse function of stimuli probability. Thus, data showed that simple RTs lengthened least and RTs to the most improbable stimulus lengthened most. This is consistent with the interpretation that the timing of basic sensory and motor processes were uncoupled by the lesions, but that the processes of response selection were profoundly slowed. Error data relevant to this interpretation as well as pre-lesion data obtained during drug probes (apomorphine (0.01, 0.3 mg/kg, sc), amphetamine (0.03, 0.3 mg/kg, ip) and haloperidol (0.1, 0.3 mg/kg, ip) will be reported.
817.15


The dorsal and ventral subdivisions of the reticulata (DL-SNR) project directly to the parvocellular reticular formation (PCRN), an oropremotor region, and to the lateral intermediate layers of the superior colliculus (LS-C), which participate in head control. Both the LS-C and PCRN receive direct projections from the lateral deep cerebellar nucleus (L-DCN). The purpose of the present study was first, to characterize in more detail anatomical connectivity from DL-SNR to L-DCN to the LS-C and PCRN, and second, to establish the role of this circuitry in a task required to maintain precise co-ordination of the head and mouth. Multiple retrograde and anterograde transport of tracers was used to investigate neural connectivity at the light microscopic level. The main findings were: (i) in dorsal DL-SNR a high proportion (5-70%) of retrogradely labelled cells were double labelled following the injections of fluorescein isothiocyanate into L-SC and PCRN. (ii) In L-DCN segregated populations of single labelled cells were found following similar injections of fluorescent tracers into L-SC and PCRN. (iii) Simultaneous injections of PHA-L into DL-SNR and biotinylated dextran into L-DCN produced intermingling of anterograde target structures in label in L-SC which surrounded cells retrogradely labelled with cholera toxin B injected into PCRN. An injection of 50% of 4 Formalin into the hind paw of a rat produces a reliable biphasic response in which the animal turns to lick and bite the injected paw. This response was provoked in a dose dependent manner by bilateral injections of the GABA agonist muscimol into the L-SC (12.5-50ng; 0.5/side). Interestingly, the animals’ attention and activity were redirected from lower to upper regions of space when doses of muscimol >20ng were injected into L-SC. These data suggest that: (i) premotor circuits controlling the head and mouth receive converging input from both dorsal tegmentum and (ii) the integrity of this circuitry is essential for effective performance of a task requiring movements of the head and mouth.

Supported by Wellcome Trust grant 0306/199 to FR

CEREBELLUM: GENETIC MODELS

818.1


In the genetically dystonic (dt) rat, a model for torsion dystonia, the deep cerebellar nuclei (DCN) have been identified as a site of abnormality. This study used extracellular single unit recording and microiontophoresis to test the sensitivity of DCN neurons to inhibitory input. Under urethane anesthesia, GA(A 0.1M, pH 4.5), taurine (0.5M, pH 3.0) and bicuculline (5mM, pH 3.0), the GABA antagonist, were applied to DCN neurons in dt rats and unaffected littermates, aged 18-25 days. In littersmates, all DCN neurons tested (n=17) were inhibited by GABA. Inhibition was defined as a reduction in firing rate of at least 50% at injection currents of 90 nA or less. In the dt rats, only 79% of the neurons tested (n=24) responded. Unresponsive cells were located mainly in the n. interpositus. In responsive cells, reduced sensitivity in comparison with littermates was noted in the lateral nucleus. In littersmates, 56% of the neurons tested (n=18) were inhibited by taurine. In dt rats, however, only 23% of the cells tested (n=22) responded. All cells tested showed increases in firing rate to application of bicuculline. These results are consistent with studies showing decreased binding of ['H-muscimol in the DCN of dt rats and indicate reduced inhibitory control in the dt DCN. (Supported in part by the Dystonia Medical Research Foundation.)

818.2


The complete loss of Purkinje cells (PC) in Purkinje cell degeneration (PCD) mutant mice removes the major inhibitory input to the deep cerebellar (DCN) and vestibular nuclei (VN). Neuronal activity, however, is not enhanced in the VN, suggesting compensatory reactions of other inhibitory neurons, but the total number of GABAergic cells is not increased and the density of GABAergic terminals is greatly reduced compared to wildtypes. In the present study we show that the number of Parvalbumin-immunopositive (Parv+) somata, which are absent in the DCN and at a very low level in the VN of normal wildtypes, increases massively in these nuclei of PCD mutant. This increase is closely correlated temporally and spatially with the PC loss. In addition, Glycine-immunopositive (Gly+) neurons are drastically more packed in the DCN of PCD, and even after correcting for nuclear shrinkage (>50%) the total number of Gly+ cells remains significantly higher by 1/3 compared to wildtypes. Moreover, the size and density of Gly+ terminals contacting DCN neurons are increased in PCD. Double labeling shows that Parv+, which often colocalizes with GABA, is highly localized in small and medium-sized Gly+ neurons of the DCN and VN in PCD mutants. Taken together, the increase in Parv+ neurons that mainly colocalize with Gly and in terminal size and density of Gly+ small and medium-sized neurons reflect most probably an enhanced inhibitory activity and, as these are presynaptic to the large output neurons, a reduction in turn of the activity of the latter. This mechanism is likely to compensate for the PC loss and contribute to the mild motor deficit in PCD mutants, as a somatally Parv+ increase can also be evoked in single subdivisions of the DCN and VN by discrete cerebellar lesions in wildtypes, and in Weaver mutants where motor impairment is improved by this operation.

818.3


Three month old Shaker mutant rats are characterized by coincident degeneration of lobule I-X Purkinje cells and motor ataxia. Inversion was used to study the expression of c-fos activity in the cerebellum from Shaker mutant and normal rats. c-fos immunoreactivity was observed in the cortex, white matter, and deep nuclei in mutant rats. In the cortex clusters of Fos immunopositive cells were located in the granule cell layer of lobules I-X. There were no immunopositive cells located in other cortical layers. Subjacent to the clusters of Fos positive cells in the granule cell layer Fos immunopositive cells were frequently present in the white matter. In other areas clusters of positive cells were located in the white matter at the base of folia and lobules. Fos immunoreactive cells were present throughout the cerebellar nuclei. These cells were more numerous and grouped together in regions deafferentated of inhibitory corticocerebellar input. Comparable patterns of Fos immunoreactivity were not observed in normal cerebellum. These findings of increased cerebellar activity in Shaker mutant rats could be the base for the ataxia and tremor seen in these animals. (Supported by NIH grants RO07013 and NS20227).

818.4


The shaker mutant rat spontaneously demonstrates Purkinje cell and inferior olive degeneration (LaForge et al., Lab. Animal Sci. 42: 19-26, 1992). Clinically shaker rats are classified as strong shakers or mild shakers depending on the presence or absence of tremor. We were interested in determining whether gait deficits in the strong shaker mutants correlated with the described progression of Purkinje cell degeneration (Tolbert et al., J. Comp. Neurl., in press). At regular intervals between 1-6 months of age, we evaluated gait by having normal and shaker rats traverse a clear plexiglass tunnel. Hindfeet were marked with black ink and forefeet were marked with red ink for footprint identification. Measurements of stride length, step length, and stride width were made. Spatial distribution of hindlimb footprints relative to ipsilateral forelimb footprints were measured. Landmarks on the hip, scapula, wrist, and ankle were marked with black ink. Left forelimb- and hindlimb kinematics were videotaped and analyzed using Peak Performance. At 1 month of age, shaker gait appeared normal. By 3-4 months of age, walking track analysis of shaker gait revealed decreased step and stride length, increased hindlimb stride width, and increased variation of hindlimb placement relative to ipsilateral forelimb placement. Video analysis of kinematic data revealed abnormalities showed forelimb high stepping, highly varied ankle path during hindlimb swing phase, larger and more variable hip displacement, increased time spent in stance phase, and disturbed temporal and spatial patterns of footfalls. Hindlimb plus revealed a lack of temporal synchrony in both the lateral sequence and diagonal copulation that characterize normal rat gait. At 6 months of age these deficits are more pronounced. In addition, the rats fell more frequently and took extra footfalls uncoordinated with footfalls of the ipsilateral limb. These results are consistent with the described time course and extent of Purkinje cell degeneration in shaker rats (Tolbert, ibid). (Supported by the Program in P.T.)
818.5
INFERIOR OLIVARY NEURON NUMBER IN DEVELOPING NORMAL AND LURCHER MICE. J.A. Heckman*, Department of Anatomy and Neurobiology, St. Louis University Medical School, St. Louis, MO 63104-1028.
The number of neurons which comprise the inferior olivary complex has been estimated to be 70,000 in the wild-type and 30,000 in the mutants at 10, 20, and 30 days of age. Current results reflect neuronal number estimates in three mice of each genotype at each age. Serial 50μm frozen sections were stained with cresyl violet and analyzed using a Neuruloc® system. Boundaries were drawn around each of four olivary subdivisions (caudal and rostral medial accessory olives, dorsal accessory olive, and principal olive) in every other section, and these outlines were used by Neuruloc® to generate volume estimates of each subdivision. Neuron density in each of the subdivisions was estimated by counting the number of neuronal nuclei within a randomly positioned 125,000 μm³ cube of tissue in each section. Average density values were multiplied by the volume to provide neuronal number estimates of each subdivision. The number of neurons in the wild-type inferior olive remains relatively constant (about 13,000) over the period examined. The number of lumber olivary neurons is already reduced by about 30% at 10 days of age, and falls to about 30% of normal by 30 days. Although a previous study demonstrated homologous olivary atrophy in adult lurcher mice, the present results suggest a differential rate of loss from the olivary subdivisions. Most notably, the current data suggest a relative sparing of neurons in the caudal medial accessory olives during the period investigated. This work was supported by NINDS grant NS33969.

818.7
GROOMING IN WEAVER MUTANT MICE. R. Landon* and C. Strazielle. Université de NANCY I et Université de Montréal, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal (Québec) Canada H3C 3T7.
Since electrical stimulation of the medial parts of the cerebellum (vermis and fastigial nucleus) elicits grooming in normal rats, we wished to determine to what extent cerebellar atrophy affects the grooming response. Weanling mutant mice, characterized by degeneration of cerebellar atrophy affects the grooming response. Weaver mutant mice, characterized by degeneration of cerebellar granule cells and midbrain dopamine neurons, were compared to normal mice during self-grooming after water immersion. There were no intergroup differences for grooming episodes, grooming sequences, and grooming episodes per sequence. One grooming component appeared less frequent in weaver mutants: face washing and one more frequently: abdomen licking. Normal mice often closed a sequence by grooming an anterior body part on 68.9% of occasions, whereas this pattern was reduced (22.5%) in weavers.
Funded by NSERC (Canada).

818.8
Although monoaminergic (Maergic) systems can form classical synaptic junctions in many central regions, the vast majority of Maergic axon terminals are "non-junctional", which correlates with the paracrine action exerted by these systems. To assess the influence of the cellular environment on synaptic modeling of cerebellar Maergic axons, noradrenergic innervation of the wild type mouse cerebellum was compared that of the weaver (granular) cerebellum. Noradrenergic axons were ultrarapidly identified using immunocytochemistry for tyrosine hydroxylase (TH), with an antibody provided by R. Reischeidt. In wild type and weaver cerebella, almost all TH+ varicosities were non-junctional and their density was much greater in weaver than in wild type cerebellar. From the samples of TH+ varicosities, neuronal elements apposed to noradrenergic axon terminals were identified and quantified. 80% of the TH+ varicosities were apposed to dendritic profiles, of which about half belonged to Purkinje cell dendrites; 60% of the TH+ varicosities were apposed to other axon terminals. In wild type mice, 45% of the dendritic profiles apposed to TH+ varicosities were spatially contacted by apposed TH+ axon terminals, whereas in the weaver mouse this percentage decreased to 22%.
These results demonstrate that cerebellar noradrenergic innervation is almost exclusively of the non-junctional type, and that Purkinje cell axons do not appear to be the only target of this innervation. Granule cell absence does not change the fate of the noradrenergic innervation, because it does not evolve from a mostly non-junctional into a junctional input. This is in contrast to the report for serotonin axon terminals in a rat pyramid anatomy of the weaver mutation (Baudet and Soecht, Brain Res. 296:305, 1981).

819.1
SYNCHRONIZATION EFFECTS OF AUDITORY RHYTHM ON GAIT IN HEALTHY ELDERLY AND PARKINSON’S DISEASE PATIENTS. M. Hills*; T. C. McNaboh; S. E. Brown; R. R. Riege; J. A. Miller; Center for Res. in NeuroRehab., Colorado State Univ.; Center for Human Motor Res., Univ. Michigan; Posseid Valley Hospital; Port Collins CO 80223.
The effect of auditory rhythm on stride timing in Parkinson’s disease (PD) was studied in a frequency-dependent fashion. Twenty PD patients dopedependent (ON), 10 PD patients off medication (OFF), and 10 healthy elderly (HE) subjects completed 4 tasks: (1) 30 m baseline walking at preferred speed; (2) walking with rhythmic cue matched to their own cadence; (3) walking at rhythmic cue 10% faster than baseline; (4) walking with rhythm faded. Rhythmic signal frequency entrained gait cadence across all frequencies in subjects existing OFF medication and exhibiting continuous step-by-step phase drift. Rhythmic cue decreased variability of stride intervals (RI) in the OFF-Group by 62%, and between 10 and 30% in HE and ON-GP groups. Rhythmic cue was intrinsically interval dependent and HE closely matched across all frequencies. OFF-Group patients had larger synchronization errors (SE) (matched cue: 125 m; faster cue: 142 m) than ON-PD patients (75 m; 98 m) and HE subjects (72 m; 92 m). Phase angle transformations of SE to normalize for ISI differences showed the same results. SE variability was similar between OFF-OPD and ON-GP groups (40.5 vs 47.3 ms) and breath was generally faster in both groups (45.9 vs 48.2 ms). We conclude that timekeeper entrainment occurred in the HE and both PD groups, however with larger and more variable synchronization offsets in the OFF-Group.

819.2
The purpose of this study was to examine the ability of patients with unilateral cerebrovascular accidents (CVAs) to utilize kinetic feedback to modify pedaling techniques. The relationship between performance and neurophysiological parameters such as sensation, proprioception, kinesthesia, and cognition were explored. Eight male subjects with unilateral CVAs were randomly assigned to two groups. The bicycle apparatus was a recumbent bicycle with pedals capable of measuring normal and tangential components of the applied force. Training consisted of twelve one-minute trials with one minute rest between trials, three times a week for each subject. Feedback consisted of visual and verbal feedback regarding patterns approximating the effective force bilaterally after each trial during the rest period. The NO-feedback (NF) group received no feedback. No feedback was given during the pre/post testing sessions. Effective pedaling was determined by the amount of positive crank work involved/noninvolved (ON) leg ratio. The NF group showed improvement in this parameter, but the HI group did not. Possible explanations for the lack of improvement in the FB group are: 1) the cyclical nature of cycling allowed for natural patterns to develop without feedback, 2) with feedback, frequent trial-to-trial changes interfered with the development of balance patterns, 3) the feedback may have been too complicated for this population, and 4) development of error-detection mechanisms by integrating the visual feedback and kinesthetic variables was limited by the compromised learning and integration abilities. This increased in GABA-A receptors, subjects with subcortical lesions tended to perform better than those with cortical lesions. In the three most important effective pedaling parameters, the majority of the subjects who had ON ratios closer to 1.0 (complete symmetry) had subcortical lesions.

The majority of children receiving treatment for acute lymphocytic leukaemia (ALL) develop weight bearing and, in severe cases, wheelchair confinement (Halton, et al, 1993). Children with ALL admitted to the McMaster University Medical Centre are treated on the Dana Farber Cancer Institute Protocol, having a prospective rate of complete remission of 80%. Upon presentation, patients are categorized as either high or standard-risk of relapse (HR or SR) based on (primarily) total white blood cell count and age. Patients in the HR category receive 3X the dosage of corticosteroid (Dexamethasone) than those in the SR category and are cranially irradiated in addition to receiving the SR consolidation and maintenance protocol. SR males receive cranial irradiation but the standard sternal dose.

Complete, kinetic gait analyses were performed on 21 patients who were at various stages of the 24 month treatment protocol. In addition, total serum protein, creatine phosphokinase, serum 3-methylhistidine and hydroxyproline levels were monitored in an effort to assess myofibrillar and collagen degradation secondary to administration of Dexamethasone. The HR females (N=6) were the most severely affected, with the maximum hip joint power at toe-off above showing a definite decline as treatment progressed. Those least affected were the SR males (N=7) whose hip joint powers at toe-off did not show a decline as treatment progressed... SR females (N=3) and HR males (N=3) showed progressive declines in hip powers but to a much lesser extent than the HR females. Serum 3-methylhistidine and hydroxyproline levels correlated well with maximum hip powers (r=0.748 & 0.891, respectively). Knee and ankle joint powers showed no change to the same extent. These findings suggest that the observed gait abnormalities in these children could be attributed to a corticosteroid induced myopathy affecting the large proximal lower limb muscles.


Why do humans switch from walking to running as speed increases? According to the dynamic theory, the shift between gait behaviors as a bifurcation between two attractors (Diedrich & Warren, 1995). This theory predicts that the locomotion system will exhibit hysteresis, which is a tendency for the walk to run transition to be made at a higher speed than the run to walk transition. Strong evidence for hysteresis has been found in experiments where speed varied in a step-wise manner through the transition region (e.g., Freeland, 1998). In contrast, in trials in which speed was continuously varied, results concerning hysteresis have been mixed (Diedrich & Warren, 1995). Therefore, in order to clarify these findings, six participants performed transition trials in which the time scale of the change in the control parameter was varied. Specifically, transition trials were performed in the following conditions: 1) speed was continuously varied, 2) speed was changed in steps of 0.5 s, and 3) speed was changed in steps of 20 s. These trials were analyzed with respect to the direction of the transition, thus resulting in a more complete evaluation of hysteresis.


We are interested in determining how binocular vision influences the kinematics of obstacle avoidance during locomotion. Young healthy subjects (n=7) were asked to estimate heights of obstacles verbally, and following that subjects (n=4) were required to step over obstacles of three different heights (Low=0.5cm, Medium=1.0cm, High=3.0cm) during locomotion under monocular and binocular viewing conditions. Head and lower limb kinematics were monitored using an OPTOTRAK system. Verbal estimation of obstacle height was similar under two viewing conditions, although the variability in slope of the regression line between the estimated and actual height was more variable under monocular viewing. Choice of leading limb was affected by monocular viewing but only for the high obstacle: when the right eye was patched subjects preferred (approx. 2 to 1) the lead with the left limb and vice versa. Leading limb toe clearance was affected by monocular viewing, but only for the high obstacle (15.4 vs 12.7cm). Trailing limb toe clearance was higher for the monocular condition, for all three obstacle heights (11.13 vs 9.13 cm). Over 86% of transitions were made by the obstacle in the monocular viewing condition and after the obstacle in the binocular viewing condition. Supported by grants from NSERC Canada.
CONTROL STRATEGIES FOR VISUALLY GUIDED STEPPING

M.A. Hollands and D.J. Marchie-Horvat (S2PN/Brain Research Association.)

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Human subjects performing a task that requires visual guidance of each step onto irregularly placed 'stepping stones' usually locate the next target of footfall just before they lift the foot to be repositioned in time to the end of stance. When asked to negotiate the same walkway without ambient lighting, and with each stone's location indicated by a central light spot (LED), subjects' stepping and eye movements are unaltered. Under conditions of intermittent visual denial, in which all LEDs were temporarily extinguished at irregular intervals, a proportion of steps are affected, but not all, suggesting that unimpeded on-line visual feedback is not crucially important, even when (as here) each step required visual guidance. In an affected step, the effect is primarily on stance, rather than swing, and is an increase in stance duration, suggesting an effect on planning during stance of the next step (next swing towards the next target) rather than on execution of the ongoing step (current swing). Under 400 and 500 ms denial, there is no effect when the target disappears in the first 100ms of stance; this implies that crucial planning does not take place until near the end of stance of the foot to be repositioned. Accurate saccades, followed by accurate steps, to the next footfall target are often made when it is invisible. This implies that saccade generation might itself be useful even when it does not result in a focal image in the neural processing preceding a saccade to a target is used in planning the next step. We propose that a task such as this is executed under central nervous control operating as a feedforward visuo-motor control system that is robust during periods of visual denial of up to 800ms. Analysis of eye movements recorded during this experiment (currently underway) should further elucidate the underlying control strategies used in this locomotor task.

SEGMENTAL COORDINATION DURING HOPS USING ONE OR TWO FEET IN CHILDREN AND ADULTS. P.A. McKinley*, C. Assante*, B. Ambard*, and L. Pelján. McGill University, Sch. of P.O.T., & Montreal Hospital H3G 1Y5 & UPR Neurobiologie et Mouvements, CNRS, Marseille France

The main purpose of this study was to investigate the development of segmental coordination during single hops using one foot or two legs, in two groups of 25 subjects (5.5-7.75ys) and adults (n = 6/group). Kinematics of the legs, trunk and head, kinetest at takeoff and landing, and EMG of the trunk, lower limb and neck were collected using an integrated motion analysis system (ELITE, BTS). Results were analyzed separately for 3 phases - takeoff, flight and landing - and indicated the following: 1) for head/trunk control, the children differed from adults, but not from each other, while at the lower limb, the three groups used different modes of advancing the body; 2) children use significantly more trunk and head motion to advance the body during bipedal hops and as compared to adults; 3) head stabilization in space is phase dependent, while trunk stabilization is phase independent in all subjects. It is suggested that poor timing of, and lack of adequate propulsion during the takeoff phase in children may be responsible for the observed differences. Similarities in head/trunk stabilization in space suggest that the trunk may contribute a stable reference frame from which balance control is organized. Supported in part by NSERC & FRSQ

H REFLEX MODULATION IN TIBIALIS ANTERIOR DURING PASSIVE PEDALLING. J.D. Brooke, W.E. McIvor, M. Miki, W.R. Stanes and J.E. Misserh. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, Canada N1G 2W1

Soleus H reflex gain is strongly modulated, leading to marked attenuation, consequent to passive movement of the human leg. We addressed the lack of information about any other H reflex of the leg with such movement, hypothesizing that attenuation also occurs for tibialis anterior (TA), in antiphase to the firing pattern of this muscle shows during active pedalling. With eight subjects, thirty TA H reflexes were collected at four equispaced phase positions with two passive movement velocities (20 and 60 rpm) of the pedal crank. M waves indicated stimulus symmetry. TA was tonically contracted throughout the cycle, at 5-15% of the EMG for a maximum voluntary contraction. No significant movement-induced attenuation, or phase modulation of the reflex, occurred for either velocity. Mean maximum depression was 19% of controls, at full extension (p > 0.05). This lack of modulation for the TA H reflex contrasts with typical reductions of up to 80% of control for soleus. Such marked contrasts in sensorimotor gain control between the la autogenic pathways for muscles of the human leg may indicate functional differences in the spinal reflexes they serve. Supported by NSERC (Canada).

H REFLEX MODULATION DURING REVERSE PASSIVE PEDALLING. S.P. Dukeliev, J.D. Brooke, K.B. Adams, J. Cheng, W.R. Stanes and J.E. Misserh. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, Canada N1G 2W1

The gain of the soleus H reflex attenuates to the rate of passive movement of the human leg. The attenuation is maximal at the end of leg flexion. In this phase, maximum flexion of the hip and knee occur at different positions. We asked, 'Will the gain changes still occur when the leg is moved backwards?'. We found that the H reflex attenuation was the same as that when the leg was moved in the forward direction.
819.15


Gating of SEPs becomes more pronounced with increased movement velocity. Also, the gain of the soleus H reflex correlates with the rate of tissue stretch around the knee during passive cycling. We hypothesized that SEP gain would be similarly dependent on the rate of stretch of the knee extensors. SEPs from Cr2 and Cr3, referenced to Fpz, along with soleus H reflexes were elicited by electrical stimulation of the tibial nerve at the popliteal fossa in 6 subjects. Combinations of rates and ranges of cyclical passive movement of the right leg were used to obtain two sets of three conditions with equivalent estimated rates of stretch of the knee extensors (4 and 16 mm/s). SEP's sampled during passive movement leading to the greater estimated rate of stretch were significantly depressed compared to those sampled during the lower rate of stretch and the stationary controls (p<0.05). The maximum attenuation across all conditions was 40% of the stationary controls. There was also a significant effect (p<0.05) between the conditions with equivalent rates of stretch. Thus, it appears that, at least for mild attenuation, the controls for SEP gain read both movement range and rate, and not simply the rate of extensor muscle stretch. Supported by Nserc (Canada).

819.16


In a previous study we described an experimental paradigm which adaptively remodels non-visual control of curvature in the trajectory of forward locomotion in such a way that when trying to walk straight ahead, blindfolded subjects inevitably described strongly curved pathways (Gordon et al 1995, EBR 102). In the present study we chose "stepping-in-place" rather than forward locomotion as a means of measuring long-term post-adaptive effects. Six normal subjects participated in 7 trials each, during which they "stepped-in-place" (i.e. without turning relative to space) on the center of a horizontal disc which was turning at rates ranging from 11.25 - 90 deg/sec and for durations ranging from 7.5 - 60 min. Following the procedure, blindfolded subjects attempted "stepping-in-place" on the stationary floor. Results: Post-adaptation all subjects turned relative to space without any perception of rotation, the direction of turn being the same as that of stepping relative to the turning disc. The initial response (up to 20 deg/sec) was linearly related to the velocity of the turning disc (slope=0.41, r=0.79) up to 45 deg/sec after which the response plateaued. The initial response was independent of stimulus duration over the range tested. Two additional response characteristics were: 1) an early (first min) depression (likely due to vestibular stimulation), and 2) a subsequent response decline best fitted by a double exponential curve of short and long time constants on the order of minutes and hours respectively. Presumably this novel, adaptive system normally serves to maintain proper calibrations in the non-visual, ground-based control of locomotor trajectory.

Supported by the T. Rose Foundation, MRC-57503, Alberta Heritage Foundation for Medical Research.

819.17

A SEX DIFFERENCE IN HUMAN TURNING BIAS. L.A. Mead* and E. Hamson. Dept. of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5B7.

Extensive research with rodents has clearly established the existence of individual differences in the preferred direction of spontaneous and induced rotation. Sex differences also exist, in that female rats demonstrate stronger rotational biases than males. In an attempt to generalize these findings to humans, the "human rotoimeter" has been developed. It is capable of measuring spontaneous rotational movement in humans (Bracha et al., 1987). We report here a new method for measuring turning bias in humans which is fast, reliable, and performs in the controlled laboratory condition. Four tredpads were arranged on tables surrounding an open square area, 3 m across. Every 5 s, one of the tredpads emptied a 1 s tone, to which subjects responded by approaching the tredpadd and checking a response sheet. Half of the tredpads in which the tone was heard from directly behind the subject. The experimenter recorded the direction the subject turned on each trial. Sixty-one right-handed, medication-free subjects (35 males, 26 females) were tested using this paradigm. On average, both males and females demonstrated rightward turning biases on the critical trials, however the tendency to turn to the right was significantly stronger for females. Among 43 subjects who returned for a second session, we found a significant correlation of 0.77 between the turning biases measured at the two sessions. These findings support the existence of a sex difference in turning bias in humans, and suggest that the rotation task described here is a reliable and valid method of measuring this bias.

819.18


The anatomic architecture of the head and neck is one of instability. However, head stability is essential for maintaining gaze during daily activities such as locomotion. In the horizontal plane during locomotion, subjects successfully maintained head stability (Crowell, Soc. Neurosci. Abstr., 1994). This was accomplished by head with respect to trunk gains <18°/° and relative phases <180° at 1 Hz. At higher frequencies, gains were now 1 and relative phases were 180° indicating a greater challenge to head stability at these frequencies. A somewhat different picture has emerged in the sagittal plane. Ten subjects walked at their natural cadence while angular velocities of the head and trunk were measured in the sagittal plane. Preliminary results of four subjects showed average head to trunk velocity of 60.4 % toward flexion and 42.1 % toward extension. Frequency analyses demonstrated a frequency range of .24 to 8.3 Hz related to this motion. Subjects showed some ability to control trunk gains near 1 and relative phase values of 180° at all frequencies with few exceptions. These frequency and velocity characteristics indicate that the challenge to head stability in the sagittal plane is greater than that of the horizontal plane. Therefore, even at low frequencies in the sagittal plane, head motion perfectly compensates trunk motion in order that head stability is maintained. In the sagittal plane, gravity exerts a greater effect as the head increasingly deviates from vertical. The larger average peak velocity in the flexion direction is an indication of the gravitational effect on head motion in this plane. Supported by grant DC01125.

COGNITION XIII

820.1


In healthy elderly subjects, Alzheimer's patients and other patient populations, glucose ingestion enhances performance on several tests of memory. Similar findings in young adults, however, have not been evident. Perhaps the tests used to demonstrate facilitation of memory in aged and other populations lack the sensitivity or difficulty needed to observe glucose enhancement in young subjects. In the present study, we addressed whether glucose facilitates memory and attention in young adults on memory-related tasks. A counterbalanced, crossover design was used, with each subject tested on 2 occasions: after glucose (50 g) or after saccharin ingestion. A series of cognitive tests was administered to assess memory, working memory capacity, and selective attention. Six normal adult males were tested. Memory was measured by words (Squire and Zouzounis, 1993), working memory for words (Salthouse, 1992) and attention (Minnesota clerical number checking test). Significant enhancement of performance on the memory tasks (impaired performance on the glucose condition) due to glucose was found on both the immediate and delayed recall of the narrative prose passage. Scores on narrative prose and attention tests were positively correlated with peak glucose concentration after glucose consumption. Cognition was also assessed on completion correct items in the attention test, but only when blood glucose rose to levels greater than 60 mg/1 above baseline. These data suggest that glucose enhances cognitive performance in young subjects when both task load and blood glucose levels are sufficiently increased. In addition, under some conditions, glucose enhances performance on tests that emphasize attention as well as memory. Supported by ONR (NO001489-11216), NINDS (NS32914) and NIA (AG07848).

820.2

ODOR IDENTIFICATION IN HUMANS BEFORE AND AFTER RESECTION FROM A TEMPORAL LOBE. M. Jones-Gotman* & R.J. Zatorre. Montreal Neurological Institute and McGill University, Montreal, Quebec, Canada, H3A 2B4.

Mild deficits in odor identification are observed after temporal-lobe resections in patients tested binohally (Jones-Gotman & Zatorre, Neurpsychologia 26, 387-400, 1988) and monohinally (Jones-Gotman et al, 13, 1982) and using the University of Pennsylvania Smell Identification Test (USIT). In this study, we investigated the contribution of possible pre-existing deficits to performance on the USIT in 46 spinal patients tested monohinally before and after excision from the left or right temporal lobe. Results showed impairments in both groups before surgery without significant difference between the nostrils, and an overall decline in the left-resection group after surgery. The right-resection group, however, showed a slight gain rather than a loss after surgery. The post-surgical loss in left-resection patients underlines the strong verbal component in this olfactory task. The preoperative deficits in both groups are too specific in either temporal lobe is sufficient to disrupt normal odor identification. Funded by MRC MT-10341 to M-JG and RZ.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
820.3 THE DEVELOPMENT OF FACE DISCRIMINATION CONTINUES INTO ADULTHOOD: AN ERP STUDY. I.D. Alvarez* and R.L. Neville. UCSD Dept. of Neuroscience and Cognitive Science, La Jolla, CA 92030.

Clinical, behavioral and electrophysiological studies have led to the proposal that, in adults, specialized mechanisms within the right hemisphere are preferentially involved in the discrimination of faces. Moreover, several studies suggest that adults use these specialized systems in the discrimination of upright but not inverted faces. Comparatively little is known about the developmental time course of the neural and cognitive substrates underlying face discrimination. While some studies suggest that a specialized mechanism for face processing exists even in newborns, behavioral studies have shown that children may not use the same mechanisms as adults in face discrimination tasks. For example, it has been reported that children do not show better performance on upright than for inverted faces, or the left visual field advantage for upright faces seen in adults. These studies have suggested that this adult pattern emerges relatively late in development, if at all.

The present study used behavioral and electrophysiological techniques to examine the development of face discrimination from the age of 9 to adulthood. Event related potentials (ERPs) were recorded from 9, 13, 16 year old, and adult subjects performing a face matching task.

Behavioral findings showed that performance on the task improved with age, and was better for upright than for inverted faces in all age groups. ERP data provided clear evidence of major developmental changes in the processes underlying face discrimination over the age range studied. As in adults, ERPs recorded from children displayed a negativity around 250-450 ms to the second member of a pair of faces. This negativity was greater for mismatched than for matched faces. However, the ERPs of the child groups did not display the hemispheric asymmetry or the difference between upright and inverted faces that was observed in adults.

These results suggest that the adult pattern of face discrimination develops gradually, and appears at a much later age than previously believed (>16 years).

820.5 PERCEPTION OF COMPOUND VISUAL STIMULI AND HEMISPHERIC SPECIALIZATION IN HUMANS AND BABOONS. J. Fagot, J. Requin* & C. Deruelle. CNRS, Lab. of Cognitive Neurosciences, Marseille, France.

A matching-to-sample task was used to assess the ability of baboons (n=8) and humans (n=14) to process the global and local information of compound visual stimuli. During the test, a compound stimulus made of a large square made of smaller circles, was displayed for 120 ms in the left (LVF) or right (RVF) visual half-field. Sample presentation was followed by the display of two comparison forms, one matching the sample at either the global or local level, the other being neutral. By releasing a joystick, the subjects had then to select the comparison form matching the sample. The two species exhibited a LVF (right hemisphere) advantage for global matching, and a reversed but insignificant RVF (left hemisphere) advantage for local matching. In humans, scores were better and speed faster, when matching had to be made at the global level. In contrast, baboons showed a significant score and speed advantage for local matching. This species difference was replicated in 2 additional experiments, in which effects of stimulus element sparsity and subject familiarization with the forms were assessed. Overall, results suggest (1) that global dominance is not mandatory in nonhuman species, and (2) that this effect in humans has some strong cognitive, rather than purely perceptual (peripheral) bases.


When navigating, rats use distal, spatial cues to construct a spatial cognitive map (O'Keefe & Nadel, 1978). This map facilitates navigation and is apparently constructed even if the animal has never physically navigated through the entire environment. Recently, this idea has been challenged by Sutherland et al. (1987) who report that rats trained in the Morris Water maze with barrier restricting their exploration were not able to escape from a novel start location that was located within the restricted region. However, their study included severe alterations in the environment on the test day that may have impaired performance merely by a loss of the generalization decrement. The current study shows that when the rats are trained in the water maze with barriers are that are faded after the animal learns the task, escape performance is not impaired when the rats are started from a novel start location. However, when the barriers were not faded during training and were removed during testing (a method similar to Sutherland’s), the animals performance was impaired. Hence, the spatial cognitive map can be used to support spatial navigation regardless of the level of exploration.


Attentional models of time perception assume that temporal judgments are based on mechanisms that depend on attentional resources. In order to better understand these mechanisms, slow brain potentials were recorded during different temporal tasks. The role of the correct responses was compared to that obtained with incorrect responses to study the relation between performance and brain activity. Slow brain potentials were recorded over right central, right frontal, and parietal areas.

In the first experiment, subjects were tested on a temporal reproduction task. Four As appeared on a video screen for 3 or 4 sec and were then replaced by four Bs. Subjects had to press a button when they thought that the Bs had remained on the screen as long as the As. The As were grouped into different categories depending on their duration and three categories were compared: Accurate responses centered on the target, short responses (underestimation of 1 sec) and long responses (overestimation of 1 sec). In the second experiment, four As were presented on a video screen either with a short (2.5 sec) or a long (3.5 sec) duration and subjects had to decide if the presented duration was the short or the long one. Correct and incorrect responses were compared.

In both experiments, correct responses were correlated with a significantly lower level of activity suggesting that efficient temporal processing is related to reduced prefrontal activity. The relation between the subject’s performance and the level of brain activity suggests that the errors correspond to increased "cortical noise".

820.8 NEURAL SYSTEMS IN TEMPORAL INFORMATION PROCESSING: THE BASAL GANGLIA AND THE CEREBRAL HEMISPHERES. D.L. Harrington, K.Y. Haaland, and W.D. Stoffers. Veterans Affairs Medical Center and University of New Mexico, Albuquerque, NM 87108.

Controversy exists about whether timing is localized (i.e., cerebellum) or is a distributed process regulated by many neural systems. The present study examined the role of the cerebellar hemispheres and the basal ganglia in timing, as these neural structures have been linked to timing functions. Two cortical (RH) and 20 hemispheric (LH) stroke subjects, 30 subjects with Parkinson’s disease (PD), and 43 control subjects were studied using procedures of Keefe and colleagues (1985; 1989) for isolating timing operations. Subjects completed two motor timing tasks (1), a constant 300 or 600 ms interval task (IT), two perceptual timing tasks (i.e., a 300 and 600 ms SOA between tones), and an auditory processing speed task (i.e., vowel perception). The total variability in motor timing was partitioned into clock and motor implementation sources of variance using the methods of Wing and Kristofferson (1972). The PD group was impaired in the clock, but not the motor source of variance in the motor timing task, regardless of the IT. Only one component of motor timing was impaired in the stroke groups, but a hemispheric asymmetry was found such that the RH group showed the greatest clock variability at the 600 IT and the LH group showed the greatest clock variability at the 300 IT. Perceptual timing was impaired in all patient groups. Only the two strong frequency perception control task. The results implicate the basal ganglia and/or the thalamocortical circuits in timing. In addition, the findings are consistent with models that propose hemispheric biases for processing frequency information, and this asymmetry is further explored in analyses of intrahemispheric lesion location.
Cognition

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6-OHDA
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6-OHDA
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An
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Evidence
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or
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audiotape
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pain-induced
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6-OHDA
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of
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282.10
A THEORY OF PERCEPT PROCESSING I.D. Paradigms* L.D. Paradigms Dept
of
Biology, Univ. of
Memphis, Memphis, TN 38152 and Dept. of
Physiol.
Univ. of
New Mexico, Albuquerque, NM 87131

Any
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The
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Society
for
Neuroscience,
Volume
21, 1995
821.3 HETEROGENEITY OF PRESUMED DOPAMINE NEURONS IN THE VENTRAL Tegmental Area IN AWAKE, UNRESTRAINED RATS. \(E. A. Krieger^{1,2}, G. V. Rebec^{2,3}\), Prog in Neural Science, Indiana University, Bloomington, IN 47405.

Monoamine-containing (DA) neurons play a pivotal role in the regulation of behavioral processes. Although much is known about these cells in anesthetized and in vitro preparations, information on the mechanisms regulating their activity under natural conditions is limited. To address this issue, single-unit recording combined with microiontophoresis was used in awake, unrestrained rats to study the electrophysiological responses of neurons in the ventral tegmental area (VTA) and their responses to DA and glutamate (Glu). In contrast to the traditional dilution of VTA units into two groups – DA and non-DA cells (long vs. short spike and low vs. high rate of activity) – we found substantial variability in both spontaneous impulse activity and responsivity to the applied compounds. Although an analysis of cell types of VTA neurons, two of these appeared to be DA-containing. Type I cells, located in lateral and deep areas of the VTA, had a relatively low rate of tonic spikes, low rate of non-sustained spontaneous activity with minimal changes after presentation of simple activating stimuli and/or during movement. These cells were highly sensitive to DA, but the threshold for the Glu response was high, and the magnitude of Glu-induced activation was small with profound rebound inhibition. Type II cells, localized throughout the dorso-medial areas of VTA, had a triphasic spike of shorter duration and discharged with a bursting pattern at a highly variable rate. These cells typically showed powerful activation characterized by bursting, decreasing spike magnitude and, on occasion, episodes of depolarization afterhyperpolarization, after stimuli presentation and during spontaneous mouse behavior. Type II cells were highly sensitive to Glu-induced activation and consequent depolarization block, but the threshold for the DA-induced inhibition was much higher than for the Glu. Differences in the neurons underlying this heterogeneity are known, VTA DA cells of both types appear to be governed by strict self-regulatory mechanisms minimizing their response to different stimuli. Supported by NIDA (DA 02531).

821.5 SOCIAL REACTIVITY IN INBRED MOUSE STRAINS: MEDICATION OF FEAR BEHAVIOR BY D, AND D, DOPAMINE RECEPTORS. \(P. L. Grandjean^{1}, J. I. Porreto^{2}, J. L. Giacino^{1}, M. H. Lewis^{3}\), 1Department of Psychology, University of North Carolina, Chapel Hill, NC, 27599, 2Department of Psychiatry, University of Florida, Gainesville, FL, 32610

In previous experiments we observed that dihydroxybenzene (DHX), a full efficacy dopamine agonist with a 10 fold selectivity for D3 vs D2 sites, markedly increased social interaction in inbred ICRI mouse strains. Response to DHX was correlated with an isolation-induced increase in striatal D3 density. In this study we hypothesized that these effects would be particularly robust in inbred mouse strains characterized by high social reactivity. Given the importance of ventral tegmental activity in the expression of emotional behavior, two inbred mouse strains (AJ and C57BL/6J) high in emotionality and low and high, respectively, in motor activity were chosen. Isolated mice were administered either a D1 (DHX, 10 mg/kg) or D3 (quinpirole, 1.0-3.0 mg/kg) agonist and their behavior was assessed in a social interaction test. Both agonists induced a marked strain dependent social reactivity. In C57BL/6J mice, DHX significantly increased escape behavior whereas quinpirole (3.0 mg/kg) had only marginal effects on this measure and other measures of social reactivity. Conversely, quinpirole induced a striking increase in almost all measures of social reactivity (e.g., defensive kicking, jump, vocalization) in AJ mice. This heightened reactivity was also observed following DHX, albeit to a much lesser degree. These findings highlight the importance of dopamine receptors in the expression of fear-related behaviors. They also suggest differences in receptor subtype mediation of social reactivity as a function of strain. Supported by NSERC of Canada and MH43571.

821.7 DOSE-DEPENDENT EFFECTS OF ASCORBATE ON CONDITIONED AVOIDANCE RESPONSE. \(J. M. Guyer^{1,2}, G. V. Rebec^{1,2}\), Program in Neural Science, Indiana University, Bloomington, IN 47405.

Pretreatment with ascorbate, a known modulator of dopamine transmission in the striatum, enhances the ability of haloperidol, a dopamine antagonist, to induce catalepsy and to block the behavioral effects of amphetamine and haloperidol. \(P. Porreto^{1,2}\), Prog. Neurobiol., 43:537, 1994. In the present study, we extended this line of work to the dose-dependent version of the conditioned avoidance response (CAR) task, which is highly sensitive to changes in the CAR system. A dose of ascorbate (White and Rebec, Neurons. Protocols, in press). Increases in dopamine transmission enhance CAR performance, while decreases have the opposite effect. Adult, male rats were trained to a CAR in a pattern 15% correct, 95% correct, 50% correct, and 100% correct. 15 mm of tone onset. We tested the effects of low (200 mg/kg) and high (1000 mg/kg) dose ascorbate alone and in combination with haloperidol (0.01 and 0.05 mg/kg). Consistent with previous results, high dose ascorbate impaired CAR performance and increased response latency without altering the latency of the escape response. The low dose of ascorbic acid, in contrast, failed by itself and attenuated the detrimental effects of haloperidol. Collectively, these results confirm and extend reports that ascorbate modulates dopamine-mediated behavioral responses. This, in turn, suggests a novel mechanism by which ascorbate may enhance such responses.

Supported by NSF IBN 91-12005.

821.8 EFFECTS OF HALOPERIDOL AND SCH 23390 ON JUVENILE RAT SOCIAL BEHAVIOR: IMPLICATIONS FOR THE ROLE OF DOPAMINE. \(S. L. Buchea Yianouttos^{1}, S. Ktiri^{2}, L. Lassow^{4,2}, D. J. Salamone^{4,2} \), Department of Psychology, University of Connecticut, Storrs, CT, 06269-1020

The effects of various doses of haloperidol (HP; 0.025 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg, and 1 mg/kg (SCH; same doses) on juvenile rat social behavior were assessed. In two experiments, the effects of systemic drug administration on rough-and-tumble play (RT play) and social isolation-induced hypothermia (SI) were assessed. Social isolation-induced hypothermia in a pair of the 1MEM (or 2MEM) received an injection. Subjects were observed for 15 rather than 5 minutes, and the frequency of NP social activities (e.g., sniffing) was evaluated. Results indicate that the modified paradigm is useful in characterizing the complex nature of juvenile social behavior, as well as delineating the behavioral effects of pharmacological compounds. Haloperidol selectively affected RT play for the 2MEM sample in a dose-dependent fashion, with the 0.05 mg/kg dose resulting in excitation, and the 0.2 mg/kg dose inducing significant behavioral suppression. By contrast, HP did not significantly affect either the 1MEM sample or NP social activity at any dose administered. SCH also induced a dose-dependent decrease in RT play, and had no effect on NP social activity. Unlike HP, lower doses did not result in higher levels of playful behavior. Taken together, these results indicate that brain dopamine may be critical for the execution of RT play, but appears to be less critical for the expression of NP social behavior.


The ventral tegmental area (VTA) is a central element in a neural system that mediates the reinforcing properties of natural stimuli (such as food), brain stimulation and drugs of abuse. While VTA dopamine neurons appear to be of particular importance, the VTA also contains interneurons and non-dopaminergic projecting neurons whose role in VTA function and influence on dopaminergic neurons is relatively unknown. Using multiple chronically implanted microelectrodes, it is possible to record from many VTA neurons simultaneously. In preparation for studies of the functional interaction of VTA neurons, we have developed criteria for identification and subgrouping of recorded neurons. In the present study, bundles of 4-12 microelectrodes were chronically implanted in the VTA of male Wistar rats. Following recovery from surgery, recordings were obtained in awake and anesthetized rats during administration of drugs (a mixed D1/D2 agonist, morphine or atantilatant (μ opioid agonists), and midazan or atlan (GABA, agonists). Signifies the activity of single neurons were identified by uniformity of waveform, and decreases in probability of unit firing observed in the node of the correlation histogram. Neurons broadly classifiable as dopaminergic (long duration, irregularly shaped waveforms, relatively slow firing rates, inhibited by apomorphine, excited by μ opioid and GABA agonists) and non-dopaminergic (short duration, biphasic action potentials, excited by apomorphine, inhibited by μ opioid and GABA agonists). Furthermore, the heterogeneity observed within these two broad categories suggests that it may be possible to further subdivide these neurons, for instance into non-DA interneurons and projecting neurons, and also into subtypes of DA neurons. Supported by NIDA DA 08349 and Hahnemann University R0 90414.

821.8 EFFECTS OF L-DOPA, METHAMPHETAMINE AND APOMORPHINE ON SHUTTLE AVOIDANCE LEARNING IN NEONATALLY 6-OHDA-TREATED RATS. \(M. Takasuma^{1,2}, T. Okachii^{1} \) and L. Iwatsuki^{1}\), 1Yamano College of Aesthetics, Tokyo 192-03, Japan; 2Institute of Psychology, Univ. of Tsukuba, Tsukuba 305, Japan.

Neonatal depletion of brain dopamine produces various behavioral deficits including hyperactivity as well as learning impairment of active avoidance task (Takasuma et al., 1995). To investigate the drug effects on shuttle avoidance learning in the dopamine-depleted rats, L-DOPA (5 or 25 mg/kg, i.p., pretreated with benzerazine), methamphetamine (1 or 2 mg/kg, i.p.) and apomorphine (0.5, 1.0 or 10 mg/kg, i.p.) were injected before the training session. Rats were treated with 6-hydroxydopamine (6-OHDA; 35 μL of 6 mg/μL, ventricular pretreatment) or 6-OHDA (35 μL of 6 mg/μL) 4 days after birth. The avoidance training (50 trials x 3 days) began on Day 90 after 5-min open-field test. Though each drug elicited a dose-dependent alteration of activity in the open-field, none of the drugs increased the number of avoidance responses in 6-OHDA-treated group. In particular, the increase of intertrial shuffling responses after the injection of the drug was accompanied with improvement of avoidance performance. These results supported that the performance of 6-OHDA-treated rats was not affected by the activity-stimulating effect of the drugs.
821.9
EXPOSURE TO AMPHETAMINE IN VIVO LEADS TO ENHANCED AMPHETAMINE-INDUCED DOPAMINE RELEASE BY DOPAMINE NEURON TERMINALS IN VITRO. M. O'Neil, L. Wang, and P. Vezina. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

Experiments investigated the effect of prior exposure to systemic injections of amphetamine on the subsequent release of dopamine (DA) evoked by amphetamine from dopamine neuron terminals in the rat nucleus accumbens (N.Acc) and dorsal-lateral striatum (DLStr) in vitro. Different groups of rats were administered five injections of amphetamine (3.0 mg/kg, i.p.) or saline, one injection every third day. Two weeks following the last injection, rats were decapitated and brains rapidly removed. N.Acc. and DLStr. bilaterally dissected out and the tissue incubated in oxygenated sodium bicarbonate buffer. Tissue was incubated repeatedly for five minutes in 400 μL of medium and exposed in sucrose solutions to different concentrations of amphetamine (0.10-μM). Prior exposure to amphetamine in vivo led to significantly enhanced, concentration dependent, amphetamine-induced DA release from both N.Acc. and DLStr. DA neuron terminal tissue in vitro. The two groups that did not receive amphetamine were present in the medium: basal DA release in vitro was not altered by amphetamine preexposure in vivo. These results confirm and extend those of previous reports (Kotta et al., Neuropharmacology, 1985, 26, 423; Castaneda et al., Life Sci., 1988, 42, 2447). Together, these findings indicate that amphetamine acts in the cell body region of midbrain DA neurons to produce sensitization, these results support the view that exposure to such injections lead to long term changes in midbrain DA neuron function.

821.10

Repeated intermittent administration of amphetamine has been shown to produce an enhanced locomotor and nucleus accumbens (N.Acc) DA response to a subsequent challenge injection of amphetamine, when testing is done sufficiently long after the last drug injection. This study used in vivo microdialysis and measured locomotor activity in rats. The effects of MK-801 and amphetamine. This procedure was repeated five times. Two to three weeks after the last injection, all animals were given amphetamine (1.0 mg/kg, i.p.) and their locomotor and N.Acc. DA response measured. The S-A rats showed a significantly greater locomotor and N.Acc. DA response in comparison to S-S rats, reflecting sensitized responding by S-A animals. In contrast, the locomotor and N.Acc. DA response of M-A rats did not differ significantly from those of M-S rats. These results suggest that NMDA receptor activation is a necessary component of the development of sensitization to amphetamine. Interestingly, both the locomotor and N.Acc. DA responses of animals having been preexposed to MK-801 were higher than those of S-S rats. This may reflect an additional and separate long-term effect of exposure to this NMDA receptor antagonist.

821.11

Repeated exposure to a variety of direct (NPA) or indirect (amphetamine) DA agonists produces sensitization in young and adult rats. NMDA antagonists (dizocilpine; DIZ) block the induction of sensitization in adults, suggesting that DIZ and the DA agonists AMphetamine and NPA share a common locus of action. To further assess this possibility, we attempted to determine, using the preweaning model, whether (1) DIZ would block the sensitization induced by AMphetamine and NPA; and (2) whether DIZ, AMPH, and NPA are capable of inducing cross-sensitization. 17-day-old rats were injected on four consecutive days with DIZ (0.3 mg/kg) or saline. After 30 min, rats were then given AMphetamine (2.5 mg/kg, NPA (1.0 mg/kg), or saline. A final test occurred 2 days later, with activity, sniffing, and rearing being measured after AMphetamine, NPA, or DIZ treatment. The results showed that pretreatment with DIZ blocked the sensitization induced by AMphetamine and NPA. Curiously, DIZ, AMPH, and NPA did not induce cross-sensitization in the 17-day-old. For example, repeated treatment with AMphetamine had no effect on the behavioral actions of DIZ or NPA. Thus, these results show that DIZ, AMPH, and NPA are each capable of inducing sensitization, but that the sensitization produced by these drugs may be mediated by different mechanisms.

821.12
CUES SPECIFICALLY UNPAIRED WITH AMPHETAMINE ATTENUATE THE DEVELOPMENT OF LOCUSCULTOR SENSITIZATION TO THE DOPAMINE RECEPTOR AGONIST QUINPIROLE. C. J. Mathews and P. Vezina. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

Experiments were conducted to determine whether prior exposure to amphetamine enhances the subsequent development of sensitization to the locomotor effects of the D-2 dopamine receptor agonist quinpirole. Because conditioned drug effects have been shown to influence the expression of sensitization to amphetamine, the role of such effects in the development of sensitization to quinpirole was also assessed. Three groups of rats were first administered injections of either amphetamine (1.5 mg/kg, i.p.) or saline. Animals in one group (PAIRED) received amphetamine in locomotor activity testing boxes and, the following day, saline in their home cage. Animals in a second group (UNPAIRED) received saline in the activity box and amphetamine in their home cage. Animals in a third group (CONTROL) received saline in both environments on both days. The injection and behavioral testing were repeated daily. These injections were repeated every four days for a total of 10 quinpirole injections. Prior exposure to amphetamine had no effect on the subsequent development of sensitization to the locomotor effects of quinpirole. Both PAIRED and CONTROL group animals displayed a similar development of sensitization to quinpirole when this agonist was administered repeatedly. Interestingly, the development of sensitization to quinpirole over days in UNPAIRED group animals was significantly retarded relative to the other two groups. These results suggest that while different mechanisms likely contribute to the locomotor sensitization observed with amphetamine and D-2 dopamine receptor agonists, both instances are dramatically affected by conditioned stimuli specifically unpaired with drug.

821.13

Reports indicate that daily, repeated administration of selective D1 and D2 dopamine (DA) receptor agonists in lesioned rats may lead to the development of behavioral tolerance or sensitization in response to an acute drug challenge. Since sensitivity changes are dependent on dose and treatment schedule, we investigated the consequences of intermittent, repeated treatment of lesioned rats with vehicle (V), D1 or D2 DA agonists on rotation after acute D1 and/or D2 agonist challenges. Male Sprague-Dawley rats were given unilateral injections of 6-OHDA into the MFB. Following recovery, rats were tested for rotation after apomorphine (0.3 mg/kg), divided into four groups (N17.18gmm), and given 43, 8-irradiated injections of either V, the D1 agonist, quinpirole (0.15mg/kg), or V. A-55653 (0.03mg/kg) + Quin (0.05 mg/kg) (COMBO); rotation was examined periodically. After this repeated treatment phase (RTP), animals were challenged with various doses of either A-55653, Quin, or L-DOPA and rotation was measured. During the RTP, all but 4 rats demonstrated behavioral sensitization. Repeated A- 55653 led to increased acute, low doses of A-55653, but response sensitization following a higher dose compared to rats given V during the RTP. Rotation levels were unchanged compared to V following any challenge dose of Quin, but the rats were dosed. In contrast, rats given Quin during the RTP showed enhanced rotation in response to all compounds, including A-55653. The effects of repeated COMBO treatment were compound- and dose-dependent and tended to reflect the additive effects of repeated A-55653 and Quin. HPLC analysis indicated that rats sustained 99% striatal DA depletions. Our unilateral quinpirole treatment leads to behavioral supersensitivity in rats following an acute injection with a D1 or D2 DA receptor agonist or L-DOPA. In contrast, repeated A-55653 treatment produces dose-dependent changes in behavioral sensitivity to itself, but not to quinpirole or L-DOPA.

821.14
CORRELATION OF STRIATAL DYNOPHRIN mRNA EXPRESSION AND BEHAVIORAL SENSITIZATION FOLLOWING REPEATED D1 AGONIST INJECTION. F.P. Bear, M.B. Harrison, and J.M. Trumpe. Department of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

Repeated injections of D1 dopamine agonists have been shown both to elicit behavioral sensitization and to attenuate dynorphin mRNA expression but the correlation between dynorphin expression and sensitization has not been established. Rats with unilateral 6-hydroxydopamine nigral lesions received 1, 2, 4, 4 daily injections of the D1 agonist SKF 38393 (10 mg/kg). Contracontrol rats were observed for 2 hours following each injection and after the last injection the brains were processed for dynorphin mRNA expression by in situ hybridization. Rotation increased as a function of number of injections with a maximal response achieved after 5 injections (repeated measures ANOVA, P<0.05). Dynorphin mRNA expression was markedly increased in the dopamine denervated striatum after 4 and 8 injections of D1 agonist with the most prominent effect seen in the dorsolateral quadrant (up 250%). Dynorphin expression and rotation both positively correlated with injection number (linear regression analysis, P<0.05) and there was a positive association of dynorphin expression and rotational response (Spearman rank correlation, P<0.05). These findings are consistent with the hypothesis that increased striatal dynorphin contributes to the enhanced motor response resulting from repeated D1 agonist injection.

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STRIATAL DOPAMINE CONCENTRATIONS FOLLOWING AN ACUTE TREATMENT WITH AMPHETAMINE IN A NOVEL VERSUS HOME ENVIRONMENT: K. E. Brown* A. Badiani, J. J. Lalley and T. E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104

The magnitude of amphetamine-induced rotational behavior is greater in rats in a NOVEL versus HOME environment (Badiani et al. 1993). We hypothesized that the environment may influence the behavioral response to amphetamine by modulating striatal dopamine concentrations. In four separate studies, however, we found no differences in dopamine concentrations in the substantia nigra between rats treated in a HOME versus NOVEL environment. These studies included: 1) the dopamine turnover ratio determined by microdialysis in a HOME environment; 2) the rotational behavior of rats treated in a HOME versus NOVEL environment; and 3) the rotational behavior of rats treated in a HOME environment with a dopamine agonist, apomorphine. In conclusion, our results failed to support the hypothesis that the environment may influence the behavioral response to amphetamine by modulating striatal dopamine concentrations.

FURTHER STUDIES ON THE ENHANCING EFFECTS OF A NOVEL VERSUS HOME ENVIRONMENT ON AMPHETAMINE SENSITIVITY. E. C. Castranova, E. J. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104

Sensitization to the psychomotor activating effects of amphetamine is greater in rats treated in a NOVEL versus HOME environment (Badiani et al. 1993), as indicated by a greater rate of sensitization (i.e., the progressive increase in amphetamine effects over consecutive test sessions). We report here on the effects of a HOME versus NOVEL environment on the sensitization of the mesostriatal dopaminergic system to amphetamine in rats. The animals were implanted unilaterally with a microdialysis probe aimed at the lateral hypothalamus and the mesostriatal dopaminergic system. As an index of the psychomotor activating effects of amphetamine, the rats received seven consecutive IP injections of either saline or 2 mg/kg amphetamine. Following one week withdrawal, the rotational response to 2 mg/kg amphetamine was compared in saline versus amphetamine pretreated animals. Although both HOME and NOVEL groups did not differ, the magnitude of sensitization was greater in NOVEL rats. In Experiment 2 a dose-effect curve (0.75, 1.5, 3.0, and 6.0 mg/kg amphetamine IP) was quantified before and after six IP injections of 4.0 mg/kg amphetamine. Sensitization was induced by a parallel shift to the left of the dose-effect curve in both groups, but this shift was of about 3 times greater in the NOVEL group than in the HOME group.

CEREBRAL HEMISPHERE-DOMINANCE INFLUENCES BEHAVIORAL RECOVERY AFTER FETAL MESCENEPHALIC GRAFTING IN THE RAT PARKINSON MODEL. C. Rosenthal1 J. Gerei2 A. Brandt3 M. Samii1 G. Whetzer2 G. Nickhah1 1Neurosurgical Clinic, Nordstadt Hospital, Haltenhofferstr. 1, 30617 Hannover 2Institute of Neuro-pathology, Hannover Medical School, Konstanty-Gutschow-Str. 8, 30625 Hannover, Germany

The improvement of spontaneous sensorimotor functions after unilateral grafting of fetal ventral mesencephalon in Parkinson patients as well as in the respective animal-models has so far been incomplete and variable. Usually, there is no correlation between graft survival and transplant survival or graft-derived reinervation. Therefore we have investigated, in some degree, the extent of forepaw function and stepping behaviour, 120 female Sprague Dawley rats were divided into left-handed, right-handed and ambidextrous animals on the basis of their skilled forelimb performance. The rats received unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway either ipsi- or contralaterally to the preferred paw. Rotational and sensorimotor behaviour was assessed post lesion and 5, 18, 32, 45 and 58 weeks post grafting. Finally, the rotational behaviour in rats with a unilateral 6-OHDA lesion in the dominant hemisphere. In accordance with previous studies, morphological parameters did not seem to correlate with behavioral performance.

INTRAVENTRICULAR INJECTION OF ANTI-DpH-SAPORIN: PRELIMINARY BEHAVIORAL EFFECTS. E. C. Castranova T. E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104

Lesions have long been used to surmise neural function from the associated changes in behavior. Anti-neuronal immunotoxins offer an efficient way to produce highly specific lesions. The objective of the current study was to examine some behavioral effects of intraventricular (i.v.) injection of the anti-neuronal immunotoxin anti-DpH-saporin (anti-DpH-Sap). This immunotoxin (IT) contains of a monoclonal antibody to the non-synthesizing enzyme dopamine beta-hydroxylase (DBH) coupled to a disulfide bond to saporin, a mouse ricinotoxin to prevent an alternative pathway. The lesions because DBH is an enzyme located in the membranes of synaptic vesicles and nerve terminals of noradrenergic and adrenergic neurons. Groups of rats (n=7) received injections into the left lateral ventricle of 0ug of anti-DpH-Sap. These dose-related lesions based on previous experiments in which we observed that the locus coeruleus could be completely lesioned with 2ug of anti-DpH-Sap while both populations were killed at 10ug. One week after surgery both groups of the IT-injected rats showed a statistically significant dose-dependent depression, in response to 1mg/kg of A5 neurones at an 10ug dose while both populations were killed at 10ug. One week after surgery both groups of the IT-injected rats showed a statistically significant dose-dependent depression, in response to 1mg/kg of A5 neurones at an 10ug dose while both populations were killed at 10ug. One week after surgery both groups of the IT-injected rats showed a statistically significant dose-dependent depression, in response to 1mg/kg of A5 neurones at an 10ug dose while both populations were killed at 10ug. One week after surgery both groups of the IT-injected rats showed a statistically significant dose-dependent depression, in response to 1mg/kg of A5 neurones at an 10ug dose while both populations were killed at 10ug. One week after surgery both groups of the IT-injected rats showed a statistically significant dose-dependent depression, in response to 1mg/kg of A5 neurones at an 10ug dose while both populations were killed at 10ug.
821.21 REMOVAL OF MEDIAL TEMPORAL-LIMBIC REGIONS RESULT IN ABNORMAL DOPAMINE NEUROTRANSMISSION IN THE Rhesus Monkey.
D.L. Keidel, K. Li, N. Lam, and R. W. Watson.
Chapel Hill, NC, Duke Univ.

We demonstrated previously that neonatal lesions of the medial temporal lobe area result in abnormal dopamine neurotransmission in the caudate nucleus in adult rhesus monkeys. The present study compared the effects of neonatal limbic lesions with similar lesions made in adult monkeys on dopamine neurotransmission using in vivo microdialysis. These groups of adult monkeys were used in the investigation: normal adult rhesus monkeys had undergone surgical removal of limbic areas including the hippocampus and entorhinal cortex in infancy (N=4), and monkeys with similar lesions made in the first 3 weeks of their postnatal life (N=3). Extracellular dopamine overflow was measured in the caudate nucleus in rhesus monkeys by implanting 22 gauge guide tubes for 125 hr. After establishing a stable baseline, a 20mM K+ challenge was given for 25 min., followed by perfusion with normal CSF for 3 hr., followed by a 2nd K+ (50mM) challenge. These samples were collected with an additional 2 hr. time period there after. The 50mM potassium challenge resulted in 56% increase in normal adults and 37% increase in monkeys with neonatal lesions. 50mM K+ challenge resulted in 505% increase in normal adults and 247% increase in monkeys with neonatal lesions. In contrast, no changes in caudate dopamine overflow were observed in monkeys with adult limbic lesions following either low or high potassium challenge. These results suggest that lesions in thccus monkeys have profound effects on caudate dopamine transmission that vary in magnitude depending on age when the lesion occurred.

821.22 EFFECTS OF DISTRACTION, DELAY DURATION AND DRUG TREATMENTS ON DELAYED RESPONSE PERFORMANCE IN NORMAL MONKEYS USING AN AUTOMATED BEHAVIORAL TESTING SYSTEM. Z. O. Sinn* and J. Schneider. Dept. of Anat. and Neurobiol. and Neurology, MCF and Hahnemann University, Philadelphia, PA 19102.

Normal macaque monkeys were trained to perform an automated delayed response (DR) task with or without cue (attention) or delay (memory) distractors, or with variable duration delays, ranging from 0.1 sec. to 30 sec. Cue distractors negatively affected task performance while delay distractors had no effect on performance. Distractors presented during response choice also had no effect on performance. Performance deteriorated with long delays (p<0.05), but performance did not significantly deteriorate with short duration of presentations. Methylphenidate further impaired DR performance with cue distractors while LY-171555 and dicyclomine had no significant positive or negative effects on DR performance. Neither methylphenidate, LY-171555, nor dicyclomine further affected performance of long delay DR trials.

Supported by grant MH-46531.

822.1 IMPACT OF PSYCHOSOCIAL STRESS ON CENTRAL ALPHA2-ADRENOCEPTORS. G. Flügge* and E. Fuchs. German Primate Center, 37077 Göttingen, Germany

Alphax2-adrenoceptors are suggested to function as autoreceptors and to regulate the activity of the noradrenergic system in the brain. We have previously shown in male tree shrews (Tupaia belangeri) that under psychosocial stress (PSS), when the noradrenergic system is activated, binding sites for the subtype non-selective antagonist 3H-rauwolscine are down-regulated. In the present study we investigated binding of the alpha2A-subtype of PSS, wintering for 21 days. After different durations of PSS by in vitro receptor autoradiography. Down-regulation of alpha2A-adrenoceptors is region-specific and depends on the duration of PSS: in the locus coeruleus, the receptor is reduced by 2, 10, and 21 days of PSS and returns to normal levels thereafter. In the prefrontal cortex, the receptor number is only slightly reduced on day 2 of PSS and is normal thereafter. In contrast, in the solitary tract nucleus and the dorsal motor nucleus of the vagus, down-regulation occurs only after 21 and 28 days of PSS. Our data demonstrate the dynamic changes in alpha2A-adrenoceptors under PSS and indicate that in different brain regions, receptor regulation underlies different mechanisms. (Supported by the German Science Foundation, SFB 406).

822.2 EFFECT OF ACUTE OR CHRONIC RESTRAINT STRESS ON BEHAVIOR AND BRAIN NORADRENERGIC SYSTEM IN WISTAR-KYOTO (WKY) RATS. S. M. Tejani-Bugt*, H. M. Zafar*, and W. P. Par*12. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104 and VA Medical Center, Perry Point, MD 21902.

We have reported that WKY rats develop more restraint-induced gastric ulcers and exhibit more depressive behavior than several other rat strains. Recently we have shown that repeated exposure to novel stressors for 21 days not only exacerbates depressive behavior in WKY rats but also alters β-adrenoceptors (βARs) and noradrenaline transporter (NET) sites in several limbic brain regions compared to Sprague-Dawley rats. The present study examined whether these effects would be elaborated when an acute stressor and a chronic stressor would demonstrate adaptation after repeated stress. Rats were subjected to a 2h supine restraint stress either for 1 or 8 consecutive daily sessions. Open field behavioral data were collected on the first day and immediately after the seventh session. Rats were sacrificed following their last session. Brains were removed, frozen and sectioned for autoradiographic analysis of 125I-pindolol binding to βARs and 3H-noradrenaline binding to NET sites in discrete brain regions. Acute stress resulted in a significant drop in body weight and an inhibition of behaviors in the open field. These effects were also sustained following chronic restraint stress. In contrast, while acute stress had no effect on the NE system, chronic stress decreased βARs in the cortex, hippocampus and caudate putamen and decreased NET sites in the hypothalamus and locus coeruleus. (Research funds from VA Medical Research Services and USPHS grant NS 31699.)


Vasopressin (AVP) within the MPOA-NE is involved in the regulation of aggressive behavior and flank marking. In females, injection of NE into the MPOA-NE inhibits the ability of AVP to induce flank marking. The present study examined whether NE injected into the MPOA-NE can alter agonistic behavior in female hamsters. Female hamsters were allowed to establish stable dominant/subordinate relationships. Dominant females were implanted with intracaudate cannulae aimed at the MPOA-NE and injected with NE 300ng in 200nl saline or vehicle in a counterbalanced order. Following injection of NE, dominant behaviors (e.g., attacks, bites) significantly decreased (Saline: 5.9±2.2; NE: 0.17±0.15; p<0.05) and subordinate behaviors (e.g., retreat, defensive postures) significantly increased (Saline: 0.33±0.14; NE: 4.0±1.63; p<0.05). No significant changes were observed in a range of other behaviors unrelated to dominance/agonistic behavior. The hypothesis that injection of NE into the MPOA-NE can regulate agonistic behavior in female hamsters was supported by NSF IBN 922202 and NIH NS30022.


The objective of this study was to determine if monoamine oxidase (MAO) activity in rats changes as a result of an aggressive interaction. Male Sprague Dawley rats (200-250g) were allowed to establish residence in large wire cages over a period of 10 days. Naive intruders (250-275g) were introduced into the resident's cage for a period of 30 min and sacrificed immediately after the interaction. During this period, 4.75±0.13 roll-tumble fights occurred. Control rats from the same group as the intruders were sacrificed without being subjected to an intruder experience. Brain sections from the level of the locus coeruleus (LC) were assayed for MAO-A, which is found in MAO-A activity in the dorsal raphe nucleus (DRN) were assayed for MAO-B. A histochemical, coupled peroxidase oxidation assay utilizing nickel enhanced DAB staining was performed (Maeda et al., Cell Mol Bio., 331-11). The amount of DAB reaction in cell bodies at the LC and DRN was digitized to provide a semi-quantitative measurement of MAO activity, with precise anatomical localization. MAO-A activity in the noradrenergic cell bodies of the LC was found to be 25% greater in intruders than in controls. MAO-B activity in the DRN cell bodies did not differ between intruders and controls. These results suggest the existence of a rapid mechanism for MAO-A activation in the LC which is triggered by the stress

Supported by ASPET, Sigma Xi, and the Smithers Foundation.)
822.5

DOSP-4 LESIONS IMPAIR ORIENTATION BEHAVIOR IN RATS. C. Yu and R.N. Holdefer*. Dept. of Biology, Hong Kong Univ. of Science and Tech., Hong Kong.

The orienting reflex (OR) is an early stage of sensory information processing seen as a decrease in heart rate and desynchronization of the EEG in response to a salient sensory stimulus, and may involve the monoaminergic LC-neoauricular pathway (LC-NE). For example, a role for the LC-NE in attention and vigilance has been hypothesized, and neurons in the LC-NE of awake animals respond best to salient sensory stimuli (Stuss et al. 1989). The stimulation of the LC-NE desynchronizes the EEG and slows heart rate (Bermudez et al., 1993; Miyawaki et al., 1991).

Rats were chronically implanted for EEG and ECG recordings. The OR was elicited by upward vocalizations in a sound attenuating chamber. Neopreneplast was depilated by DSP-4 injections (50 mg/kg, i.p.), a selective neurotoxin for neopreneplast, and tested for an OR 11-16 days after injection.

In control animals (n=9) there was an 8.5% decrease (p<0.01) in heart rate (HR) 4 s after the onset of the orienting stimulus (rat vocalizations), as compared to baseline HR (6 s before the orienting stimulus). HR decreased by a maximum of 10% (p<0.01) at 13 s after the orienting stimulus and remained significantly decreased during the 60 s testing period, although some habituation was seen. In the DSP-4 pre-treated rats (n=8) there was a small, non-significant decrease in HR during the testing period.

Central NE concentrations in control and DSP-4 treated rats were determined by HPLC with electrochemical detection. NE concentrations were reduced in the DSP-4 animals by 31% in frontal cortex, 87% in parietal cortex, and 59% in the hippocampus.

We conclude that there is an involvement of central NE in the orienting reflex of rats.

822.7

ALPHA-2 ADRENERGIC FUNCTION AND VISUAL SELECTIVE ATTENTION IN MONKEYS. P. Rill*, J.K. Hietanen. Institute of Biomedicine, Department of Physiology, P.O. Box 9, FIN-00014 University of Helsinki, Finland.

Catecholimnergic innervation of the cerebral cortex may have an important function in controlling attention. The well documented ability of alpha-2 agonists to improve working memory may, at least in part, be due to the memory being protected from irrelevant or distracting stimuli (Arntzen & Constant, Psychopharmacology, 180:159-169, 1992). This indicates that alpha-2 agonists may also have a beneficial effect on attentional functions. In the present study the monkeys (Macaca arctoides) were trained to perform spatially selective visual discrimination tasks (left/right) in a red/green colour background where the colour stimulus (green/red) and its spatial location (left/central) were relevant and irrelevant stimulus dimensions, respectively. The location of the colour stimulus was compatible, incompatible or neutral with respect to the direction of the response. The different types of stimulus presentation were presented in random order within the block of testing. The effects of medetomidine, an alpha-2 agonist, and its antagonist atipamezole were studied by comparing their effects on saline control performance separately in each stimulus condition. The role of the alpha-2 adrenergic function will be discussed in the context of visual selective attention.

822.9


The effect of the alpha1 agonist, clonidine (5, 20 or 40 ug/kg), on activity of LC neurons was studied in 4 cynomolgus monkeys while they performed a vigilance task requiring sustained focused attention (see Kubiak et al, this volume). Behavioral effects of clonidine (brain penetrance 5 ug/kg) did not affect on task performance, 20 ug/kg (im), resulted in drowsiness or mild sedation with continued but improved performance, and 40 ug/kg (im) produced strong sedation. In 3 monkeys who performed well before clonidine (<5% errors of commission or false alarms; FAs; clonidine administration reduced errors (increased B) but also increased trial-by-trial latency. LC discharge was unaffected by 5 ug/kg clonidine, but was consistently reduced by the higher doses. There was an inverse relationship between tonic LC activity and task performance after clonidine. However, if LC neurons became tonically activated the monkeys showed impairment of performance. Clonidine produced an unexpectedly strong effect in the fourth monkey. This animal exhibited hyperactive behavior throughout training (FAs ~ 30%). Clonidine (20 ug/kg, sc) produced prolonged epochs of near perfect performance (56 FAs; increased B and d), alternating with epochs of drowsiness. 2 h after clonidine, performance had deteriorated to levels and motor hyperactivity resumed. Overall, task performance correlated strongly with the level of LC tonic activity: Whereas poor performance before clonidine invariably corresponded to elevated tonic LC activity, improved performance after clonidine was associated with low-frequency LC discharge. Phasic LC responses to CS+ cues, typically small in this monkey, increased markedly after clonidine.

These results suggest that found improvements of sustained attention after clonidine, especially notable in hyperactive animals, and suggest the possibility that alterations in LC activity may be a causal factor. Clonidine will be microinjected into the LC to selectively inhibit LC neurons as a test of this hypothesis. Supported by AFOSR grant F49620-93-1-0099.

822.6

TIME COURSE OF CHANGES IN HYPOTHALAMIC AND HIPPOCAMPAL NOREPINEPHRINE DURING AMPHETAMINE WITHDRAWAL. D. M. Camp*. D.K. Delanghe and T.E. Robinson. Dept. of Psychology and Neuroscience Program, Univ. of Michigan, Ann Arbor, MI 48109.

Following the discontinuation of an escalating dose amphetamine (AMP) pretreatment regimen rats exhibited a spontaneous nocturnal motor activity that persists for 1-2 weeks. Furthermore, these transient changes in behavior are associated with depletion of hypothalamic norepinephrine (NE) (Price et al. 1991; Psychopharmacol. 1991, 107, 480-492). In the present experiment we used in vivo microdialysis to further characterize NE neurotransmission during AMP withdrawal. Following 30 days of withdrawal, basal hypothalamic extracellular NE did not differ from controls, but there was a significant enhancement in AMP-stimulated NE release in this brain region. These results are consistent with the idea that alterations in NE neurotransmission may contribute to both post-AMP withdrawal behavioral depression* and the persistent effects of AMP on behavior (i.e. behavioral sensitization).

822.8


We previously demonstrated that acute, selective manipulations of locus coeruleus (LC) neuronal activity elicited robust alterations in foetal ME EEG in halothane- anesthetized rat. Similar EEG responses are observed following small infusions of noradrenergic β-agonists and antagonists into the basal forebrain region encompassing the medial septum and vertical limb of the diagonal band (MSD) in anesthetized rat. The present studies assessed whether the EEG activation effects induced by activation of MS β-receptors are observed in the absence of anesthesia, and if so, whether concomitant behavioral effects are observed. Rats were implanted with a guide cannula aimed at MS or adjacent regions. On the day prior to testing, rats were singly housed in sound attenuated chambers. On the day of testing, a 33 gf infusion needle was inserted and attached to the cannula via the plastic sleeves and videotaping of the rat’s behavior began 30 min later. 60 min following the onset of behavioral recording, an infusion (150 nl of either vehicle or the β-adrenergic agonist isoproterenol (ISO) 25 mg/l was made without disturbing the animal. EEG was simultaneously recorded in a subset of animals. MS vehicle infusions, or ISO infusions, placed outside MS did not alter EEG measures or the amount of time spent awake compared to the preceding hour. In contrast, MS ISO infusions resulted in hippocampal/cortical EEG activation and within 3–10 min. increased the time spent awake in the hour following the infusion (5 vs. 34 min). EEG responses preceded the behavioral responses and were present at the latter portions of the behavioral response which were often characterized by brief and frequent bouts of lying down interrupted by shifts of body position and brief periods of locomotor activity. These results indicate that the LC-noradrenergic system exerts an excitatory influence on behavioral and EEG measures of arousal in the unanesthetized rat, via actions within MS.

822.10


We previously reported that performance degrades with prolonged behavior on a vigilance task, and varies over shorter epochs within the task, in association with altered LC responses to CS+ cues. We now report that LC responses to conditioned vs. unconditioned stimuli exhibit converse changes during naturally occurring drowsiness, or during inattentiveness associated with high arousal. LC neurons were recorded from 3 Cynomolgus monkeys performing a vigilance task. Animals were required to fixate a central fix spot to initiate each trial of stimulus presentation; such fixation ensured attentiveness to the task. Animals were tested during epochs of inattentiveness when the cue was presented at the beginning of each trial without cue, but not by other task events. During naturally occurring periods of drowsiness task performance deteriorated, as evidenced by decreased frequency of fixation of the cue spot, increased errors of omission (increased latency to release). During drowsiness the tonic discharge rate of LC neurons decreased and LC responses to CS+ stimuli were significantly smaller. In contrast, LC neurons were not altered during epochs of drowsiness task performance, but not by other task events. During naturally occurring periods of drowsiness task performance deteriorated, as evidenced by decreased frequency of fixation of the cue spot, increased errors of omission (increased latency to release). During drowsiness the tonic discharge rate of LC neurons decreased and LC responses to CS+ stimuli were significantly smaller. In contrast, LC neurons were not altered during epochs of drowsiness task performance, but not by other task events. During naturally occurring periods of drowsiness task performance deteriorated, as evidenced by decreased frequency of fixation of the cue spot, increased errors of omission (increased latency to release). During drowsiness the tonic discharge rate of LC neurons decreased and LC responses to CS+ stimuli were significantly smaller. In contrast, LC neurons were not altered during epochs of drowsiness task performance, but not by other task events.
823.11
A COMPUTATIONAL MODEL OF LOCUS COERULEUS INFLUENCE ON PERFORMANCE IN A VISUAL DISCRIMINATION TASK
A computational model is proposed that accounts for the relationship between locus coeruleus (LC) activity and phasic locus coeruleus (LC) activity and behavioral performance during a vigilance task: improved performance during periods of lower tonic activity together with phasic bursts after target stimuli. The model shows how the relationship between patterns of LC firing and behavioral performance can be explained by changes in electrotetroactive coupling within LC. Specifically, it demonstrates that an increase in electrotetroactive coupling results in a more synchronous (i.e., phasic) pattern of firing that is triggered by target but not by distractor stimuli, and that is also associated with a lower spontaneous (i.e., tonic) firing rate. This results in tighter coupling of neocortical NE release to target stimuli, which in turn improves task performance (a reduction in false alarms without an increase in response latency to targets). The model provides an account of the influence of LC cells and NE on performance in a cognitive task, suggesting a role for electrotetroactive coupling within LC and neuromodulatory influence of NE in selective attention.

823.12
IS A CONEXIN INVOLVED IN NEURAL CONTROL OF LUMINESCENCE IN THE CNIDARIAN BENVILLA KOELLMERII? G. Germain and N. McIntyre, Dept. Biological, Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.
Of the three classes of the ctenophora Cnidaria, Hydrosaora but neither Scyphozoa nor Anthozoa possess ultrastructurally identifiable gap junctions. We present evidence that the bioluminescent LC cell system of an anhozen, the sea panay, which is believed to be coordinated by a nerve-net but which is also adrenergically controlled via cells neighboring the light-emitting cells (photocytes), requires gap junction-like coupling to effect light emission. Gap junction blockers such as octanol and heptanol reversibly eliminated in situ luminescent responses but not those of distant animals. An anti-conexin3 monoclonal antibody (anti-5x3) loaded in permeabilized cells abolished the luminescent responses. Western blot analysis revealed the presence in sea panay tissues of a Cx43-like protein. We also visualized Cx43-like punctate immunoreactivity in some epithelial layers and in the nerv-net, with greater abundance in the luminescent endoderm. Ultrastructural observations revealed only small "close appositions" but no recognizable gap junctions. These results suggest that a Cx43-like gap junction protein exists in the sea panay and is involved in transmission of bioluminescent signaling, but does not aggregate in typical large plaques. (Supported by NSERC)

823.13
THE ALPHA1-ADRENOCEPTOR ANTAGONIST PRAZOSIN ANTAGONIZES MK-801-EVOKED STIMULATION OF MESOLIMBIC DOPAMINE ACTIVITY:

The effects of the potent alpha1-adrenoceptor antagonist prazosin were investigated on electrophysiological, biochemical and behavioral actions of the psychotomimetic, NMDA-receptor antagonist MK-801 related to the mesolimbic dopamine (DA) system in the rat. Extracellular single-cell recordings were obtained from A10 DA neurons in chloral hydrate anesthetized rats. Action potentials were led into a computer for analysis of firing rate, burst firing and regularity of firing, as assessed by the variation coefficient. In addition, levels of DA and its metabolites were measured in the n. accumbens (NAc) with microdialysis in freely moving rats. Locomotor activity was assessed in rats in an open field. Rats were pretreated with either vehicle or prazosin (0.3-1.0 mg/kg), 20 min prior to MK-801 (0.05-0.3 mg/kg) administration. Administration of prazosin 1) effectively blocked the increased burst firing, but did not affect the average firing rate of A10 DA neurons 2) antagonized the increased DA, DOPAC and HVA levels in the NAC, and 3) blocked hyperactivity, as induced by MK-801. Thus, behavioral stimulation by MK-801 is indeed associated with increased presynaptic activity in the mesolimbic DA system. Moreover, a potent and selective alpha1-adrenoceptor antagonist can specifically block this, subcortically evoked DA activity without any significant effect on basal DA release in the same region. Supported by the Medical Research Council of Sweden and the Karolinska Institute.

823.14
LONG-TERM EFFECTS OF PSYCHOMOTOR STIMULANTS ON APPETITIVE AND CONSUMMATORY SEXUAL BEHAVIORS IN THE MALE RAT. M.E. Najarro, S. Schechter, J. Pestell. Dept. Psychiatry, Concordia University, Concordia Institute for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, QC, Canada.

Amphetamine and cocaine are generally believed to enhance human sexual desire, arousal, and performance, whereas long-term use of these drugs is associated with a deterioration of sexual function. Surprisingly, these effects have not been studied extensively in animals. We examined the acute and long-term effects of amphetamine (30 mg/kg) or a mixture of amphetamine sulfa (1.5, 2.5, or 5 mg/kg) on sexual behavior in sexually experienced male rats. Amphetamine or cocaine were injected prior to tests conducted every 4 days in bivelled chambers. Acute amphetamine increased anticipatory level changes, but did not affect the number of rats that mounted, intromittent, and ejaculated. Long-term amphetamine decreased anticipatory level changes secondarily to the induction of stereotypy, and also further decreased the proportion that mounted, intromitted and ejaculated. These effects reversed during withdrawal, but declined following challenge dosages of amphetamine. In contrast, acute cocaine did not affect anticipatory level changes or the proportion of rats that mounted, intromitted or ejaculated.

823.2
NEUROPEPTIDES AND BEHAVIOR III

823.2.1
THE EFFECT OF NPY ADMINISTERED TO EITHER THE VMN OR THE PVN ON LORDOSIS AND FEEDING. C. Bauer and J.F. Thornton*, Neuroscience/Biopsychology Program and Department of Biology, College of Science, Univ. of Delaware, Newark, DE 19716.

Recently we have shown that neuropeptide Y (NPY) increases lordosis in female guinea pigs. The effect of NPY on feeding in guinea pigs has not been examined. The present experiment determined whether NPY might act on either the ventromedial nucleus (VMN) and/or the paraventricular nucleus (PVN) of the hypothalamus to affect lordosis and feeding in female guinea pigs. Ovx female guinea pigs had a cannula implanted into either the VMN or the PVN. For lordosis tests, estrogen-and progesterone-primed female rats (150 pmol NPY or saline injected intrarescally) were tested for lordosis and body wall tension. NPY significantly increased lordosis when it was administered to the VMN but not to the PVN. For feeding tests, ovsx females were infused with 100 pmol NPY or vehicle and feeding was examined. NPY administered to either the PVN or the VMN significantly increased both the number of meals eaten and the frequency of feeding. It is suggested that NPY acts at the VMN to affect lordosis and at both the VMN and the PVN to affect feeding in the female guinea pig.

823.2.2
DRINKING INDUCED BY MICROINJECTIONS OF PITUITARY ADRENLINERGIC CYCLIC ADENOSINE 3'5'-MONOPHOSPHATE (PACAP38) INTO THE LATERAL HYPOTHALAMUS (LH) OF MIDDLE-aged M. Wilgus de Paradise*, H.A. Paradise, and L. Marzecza, Department of Physiology, School of Medicine, Universidad de los Andes, Merida 5101-A, Venezuela.
PACAP38 increases CAMP levels in rat pituitary cells and sulpiride increases CAMP in neurons bearing dopamine D2 receptors also, similar behavior changes were observed when bilaterally administered in the perifornical LH (pifLH). The present study tested if bilateral pifLH PACAP38 microinjections could reproduce behavioral effects of sulpiride. PACAP38 (1 nmol/0.5 µl) administers in the vicinity of the pifLH selectively induced drinking (19.7±4.8 ml/hr) during the hour following the injection. In the same rats (n=12) sulpiride (45 nmol/0.5 µl) had no effect (7.8±1.1 ml/hr). The difference between both effects was statistically significant (t= 3.61; df=11; p<0.005).

No icteric or pifLH PACAP38 microinjections promoted drinking when injected (n=10) 1.3 mm behind the effective zone. This negative result is an evidence of the hypophagocentric-specificity of the dopamngetic effect of both drugs. The dopaminergic effects of sulpiride and PACAP38 were well correlated (r=0.921; F(1,20)= 41.5; p<0.001) suggesting that both substances trigger drinking by activation of the same hypothalamic mechanisms. These results suggest that PACAP38 in the pifLH could be an integrative neuropeptide regulating drinking behavior.
823.3
CHELOCYSTOKININ ATTENUATES GROOMING VIA AN ATYPICAL RECEPTOR. T.V. Smotherman, C.K. Hall, B. Smiles. Dept. of Psychology, Univ. of Western Ontario, London, ON, Canada.

The role of cholecystokinin (CCK) in the central nervous system is not well known to interact with dopamine in various ways. We have demonstrated that CCK-8S effectively attenuates dopamine-D1-mediated vacuous chewing movements (VMs) and stereotypy in the 6-OHDA-lesioned rat (1). Furthermore, we have shown that a clear role for the CCK-A receptor in the attenuation of VMs' but the receptor antagonists蓠the effect on grooming remains unclear. While a selective CCK-B agonist was effective in attenuating the stereotypy, the CCK-A antagonist blocked the attenuating effects of a general agonist. Similar anomalous results have been observed by others in studies of anxiety and aggression. To further explore the role of CCK receptors in the modulation of selective agonists on the attenuation of grooming by a CCK-A agonist, administration of SRF 1839 (Ser-l-lauA)-C+ to rats with Sprague Dawley rats resulted in a significant increase in grooming which was reduced to control levels by the peripheral administration of the selective CCK-B agonist CCK-8. Further studies with CCK-A antagonists, H-277, and the CCK-B antagonist, L-364,660, significantly blocked this attenuation over a range of doses (200μg/kg, 100μg/kg, 500μg/kg). SRF 1839-induced VMs were not affected by CCK-A antagonists. Further study has ruled out possible interference from anxiogenic properties of CCK-8 or direct effects of the antagonists themselves. We also examined the effects of VMs on the actions of CCK-8. Vaugany abolished suppression of both VM's and grooming. Taken together, these findings suggest a predominant peripheral, A receptor mediated effect of CCK but CCK-A may be acting at a novel receptor subtype.

823.4

Argininevasopressin (AVP) is known to play a role in the central nervous system of the 2094th fetus increases motor activity, including the relatively uncommon patterns of mouthing, licking and facial wiping. The AVP effect is mediated by V1 receptors in the brainstem and spinal cord. The effect of AVP on fetal motor behavior can be potentiated by intrabehametic (IH) injection of the V1 antagonist (8-mecapto-β-cyclopentamethylenepropiophenone/DimeTyprAng)vasopressin. These findings suggest that there are at least two populations of V1 receptors: one in the spinal cord/brainstem that has an excitatory effect and a second in the brain hemispheres that has an inhibitory effect on fetal motor behavior. Fetal responses to perioral cutaneous stimulation including the presentation of an artificial nipple are also influenced by manipulation of V1 receptors in the spinal cord/brainstem. The opposite effect and potentiates responsiveness to perioral cutaneous stimulation including oral capture and grasping of the artificial nipple. These findings indicate that AVP plays a role in regulating fetal motor behavior including responsiveness to perioral cutaneous stimulation and suggests that AVP may affect behavior immediately after birth in the context of suckling.

W75 is supported by an MERIT Award from NIH (HD 16102-11).

823.6
MODULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RELEASE BY NMDA AND SHH, RECEPTORS IN PRIMARY CULTURES OF FETAL RAT AMYGDALA. D.L. Biekle, V. Sukki, M.S. Cratty, R.W. Gehr, A.A. Kaelin, M.G. Villanueva, J. Monje. Dept. of Pharmacology and Toxicology, Department of Anatomy, WVU School of Medicine, Morgantown, WV 26506

Previous immunocytochemical studies from our laboratory have demonstrated the presence of corticotropin-releasing factor (CRF) immunoreactivity in neurons in primary cultures of fetal rat amygdala neurons. We have also previously shown Ca2+-dependent, depolarization-induced release of CRF in these preparations, and CRF release in response to the excitatory amino acid neurotransmitter, glutamate. We now have evidence that glutamate-induced CRF release is not mediated by activation of NMDA receptors. NMDA mediated release of CRF. The effects of both glutamate and NMDA were blocked by AP-5 (IC50, 30 μM) or by restoring (Mg2+ 5 mM), to 1 mM Kainic acid (EC50 0.5 μM) also stimulated CRF release, and was partially antagonized by AP-5, suggesting that its effects are mediated in part by the release of glutamate in the cell culture. Activation of 5-HT1 receptors with the agonist, 8-OH-DPAT, reduced both basal and depolarization-induced (60 M K+) release of CRF (EC50 1 mM). These studies demonstrate that (via NMDA receptors) and inhibitor (via 5-HT receptors) modulation of release of CRF from fetal amygdala neurons in primary culture. Supported by NIH (NSB-9222623).

823.7
FORCED-SWIM TEST REVEALS CYCLO(OHIS-PRO) LEVELS IN RAT BRAIN. A.E. Pageke, P.L. Lloyd, M. Chilugno, I. Sattin, Psychiatry, Medicine (Endocrinology) & Research Services, West Los Angeles VA & UCLA School of Medicine, Los Angeles, CA 90073.

Electroconvulsive seizures (ECS) elevate the levels of both thyrotropin releasing hormone (TRH) and TRH-Gly (Gly-His-Pro-Gly) a TRH precursor, in the limbic system of rats. We have reported significant correlations between results from the forced swim test (a test used to predict efficacy of antidepressant drugs and ECS) and post-ECS TRH and TRH-Gly levels in four anterior limbic regions dissected from male Wistar rats (Ann NY Acad Sci, 739, 135, 1994). Because TRH and TRH-Gly levels are readily converted to cyclo(His-Pro) (CYP), we have studied the effects of ECS and forced swimming on the brain levels of this metabolically stable cyclic dipeptide. CYP has a number of CNS effects including the induction of hypothermia, a reduction in corticosterone and suppression of motor activity which may influence, and in turn be influenced by ECS and forced swim. The rat groups were: untreated controls, swim only, ECS only, and ECS + swim. Below are the changes in CYP (ng/g wet wt.) for perfom cpx., p50, ip, estradiol (E2) and anterior cortx. (AC), (Median ± SD, p-value)

Control + ECS only (18) p

PVR<0.004

STR<0.004

ECS only

AC<0.004

Swim only

AC<0.004

Control + Swim + ECS (18) p

PVR<0.004

STR<0.004

ECS only

AC<0.004

Swim only

AC<0.004

Forced swim testing significantly decreased CYP in all 3 regions tested. ECS did not affect mean CYP levels in these regions (not shown). Mechanisms of this effect are unknown. It is known that CYP can be taken up by astrocytes. We thank Dr. Joe Jackson for the CYP antisera. Supported by VA Research Service.

823.8
THE EFFECTS OF NGF, bFGF AND CEREBROLYIN ON SPATIAL NAVIGATION AFTER BILATERAL LESIONS OF THE SENSORIMOTOR CORTEX. A. Gschwenke, V. Velickovic, J. Windisch and H. Xiong. Center of Animal Biology, Medical School, Roseggerweg 48, A-8036 Graz, Austria.

Damage of the sensorimotor cortex causes spatial navigation deficits in rats. Lesions of the right hemisphere show a significant regression, and the subsequent behavioral effects measured in a Morris water maze apparatus. bFGF, NGF or Cerebrolysin (CER) a nootropic peptide drug, were infused by osmotic minipumps unilaterally into cavities made by suction or intraperitoneally (ip). For comparison, the lesioned rats were injected with saline or CER. Animals were tested in the water tank 13 days after surgery for 3 consecutive days. The length of the trajectory to a submerged platform, as well as the speed of swimming, were measured. The trajectory of lesioned animals was significantly longer than of controls but none of the treatments changed this parameter. However, all groups treated ip and the NGF group infused centrally swam faster towards the platform in comparison to the corresponding lesioned or intact controls. The same animals were evaluated 8 months after treatments. Only FGF-treated animals showed a significant improvement in comparison to the lesioned controls. We conclude that, in the short-term, CER improves spatial memory in lesioned and intact animals whereas NGF leads to more long-lasting effects.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995 THURSDAY AM
Convergent modulation of a neuromuscular junction by neuropeptides

Due to the limitations of the page, the full context and details of the research described are not fully visible. However, it appears to involve studies on the effects of neuropeptides on neuromuscular junctions, possibly in the context of motor function and muscle physiology. The research might involve the application of neuropeptides or other exogenous substances to modulate the activity of neuromuscular junctions, with implications for understanding neuromuscular transmission and potentially for therapeutic interventions.

Additional notes:

- The research could be part of a larger study investigating the role of neuropeptides in regulating neuromuscular junctions, which are critical for muscle contraction and relaxation.
- The findings might contribute to the development of therapies for neuromuscular disorders or conditions affecting muscle function.

Overall, the research described seems to focus on elucidating the mechanisms by which neuropeptides can modulate neuromuscular junctions, potentially with implications for a range of physiological and pathological processes involving muscle activity.
824.1


Prenatal exposure to alcohol can produce permanent CNS dysfunction expressed as a wide spectrum of behavioral deficits; however, the nature and severity of this dysfunction varies greatly. Developmental timing of alcohol exposure is believed to be one important environmental factor. Using a rat model system, the present study investigated the behavioral and CNS consequences of brief episodes of alcohol exposure during varying periods within the neonatal brain growth spurt, a time of rapid CNS development equivalent to the human third trimester. Sprague-Dawley rats were exposed to alcohol (6.6 g/kg) in a binge-like manner or two consecutive days via an intragastric feeding procedure. One group was exposed to alcohol on postnatal days (PD) 4 and 5, another group on PD 6 & 7, and a third group on PD 8 & 9. A fourth group served as a caudally matched, artificially reared control, and a fifth group comprised normally reared suckled controls. Subjects were tested from PD 25-43 on a series of behavioral tasks including a motor task and a reversal learning task. All alcohol-exposed groups were significantly impaired on the motor task; however, early exposure (PD 4/5) produced more severe deficits than later exposure. An opposite pattern for deficits was observed on the learning task. On this behavioral measure, severe deficits were observed in the groups following late exposure to alcohol (PD 6/7 & PD 8/9), but not earlier. This dissociation in the temporal pattern of behavioral vulnerability corresponded with the temporal vulnerability of regional brain weight reductions. Reductions in cerebellar weight, an area involved in motor performance, were most severe following early alcohol exposure (PD 4/5), whereas reductions in forebrain weight, which includes areas involved in cognitive tasks, were most severe following late alcohol exposure (PD 6/7 & PD 8/9). Thus, the pattern of behavioral deficits is consistent with the temporal vulnerability of various brain regions. Supported in part by NIAAA grant AA05523.

824.3

BEHAVIORAL AND IMMUNOLOGICAL CONSEQUENCES OF BRIEF EMBRYONIC EXPOSURE TO NICOTINE OR ALCOHOL IN DOMESTIC POULTRY, CHICKEN. C.M. Bazi, C.A. Hueston, S.H. Cohn, K. A. Nofeldt, & J. E. Cunnick. Departments of Psychology, 1Microbiology, Immunology, Prevent. Med., 1Iowa State Univ. Ames, IA 50010-3180.

Fertil eggs of domestic fowl (Gallus gallus) were injected in six of the egg air space of either nicotine tartrate (NIC: 0.0, 0.025, 0.25, 0.25 mg/ml/kg); or ethyl alcohol (ETOH; 10, 20, 30, or 40% v/v on egg) on incubation days 10-12 NIC (0.0 mg/ml) and hatch rate and hatchling survival but NIC and ETOH amounts did not. Chick body weights at hatch were not significantly affected by either NIC or ETOH.

Indons of social status vocalizations & activity differ (nicotine immobility induction and duration), and immunologic responses (leukocyte proliferation to mitogen stimulation; CreaA, LPS, PWM; blood tymanus and spleen) were measured in 3 groups of chicks at 6 week old. For NIC exposures, 14 (nicotine immobility measures), and 17 (immunrey system measures) postnatal. On the social test neither NIC nor ETOH altered distar vocalizations but both drugs decreased activity in a dose dependent manner. On the immobility test ETOH groups required fewer inductions than controls but duration was equivalent; NIC did not affect the immobility measures.

Preliminary results suggest some significant effects of NIC and ETOH on leukocyte proliferation that depend, in part, upon dose and sex gender.

824.5

ALCOHOL-INDUCED HIPPOCAMPAL ABBREVIATED MOSSY FIBER PROJECTIONS DEPEND ON THE PATTERN OF EARLY POSTNATAL ALCOHOL EXPOSURE IN RATS. D.M. Smith and C.R. Goodlett. Department of Psychology, IUPUI, Indianapolis, IN 46202.

Continuous exposure to alcohol (EtOH) in artificially reared neonatal rats during the first ten postnatal days has been shown to produce aberrant mossy fiber projections into the distal intra- and infrapyramidal zone of mid-temporal hippocampal field CA3 (West & Humes, Brain Res., 1985). This experiment examined whether induction of aberrant mossy fibers depends on the daily pattern of neonatal alcohol exposure. Harlan Sprague-Dawley rats were randomly assigned to one of the following treatments administered on postnatal days 1-9: one of three alcohol treatments (using artificial rearing methods); an artificially reared control; or a suckle control. Alcohol was given in the three different patterns, as follows: Continuous: 1. A replication of the original West & Humes treatment received 2.8% v/v EtOH in each of 8, 15-min daily feedings (n=5); Continuous: 2 received 2.8% v/v EtOH (n=3) or 3.2% EtOH Bi-hd (n=5) in each of 12, 20-min daily feedings; Bi-hd received 2.8% v/v EtOH in consecutive 12 of 20-min daily feedings (n=5). Artificially reared controls were given feedings calorically matched to the experimental groups (total kcal=11), and controls (n=5) were nursed normally by dams. Continuous: 1 treatment produces more rapid increases in blood alcohol concentration (BAC) per feeding, compared to Continuous: 2. An large volume of EtOH is administered. The Bi-hd treatment produces a high peak BAC (~250 mg/dl) that falls to 0 before the end of the day. Rats were sacrificed on PD 45, frozen horizontal sections (35 μm thick) were processed with the Timm stain for heavy metals and evaluated microscopically for the presence of distal intra- and infrapyramidal mossy fibers. In the Continuous 3 condition, 2 of the 5 cases were found to have aberrant mossy fiber projections (West & Humes, 1985). None of the remaining groups had subjects with aberrant mossy fibers. These data suggest that the rapid and continuous presence of high BACs in the early neonatal period is critical to the development of aberrant mossy fiber projections. (Supported by AA05965)

824.6


The modulation of the GABA_B receptor by ethanol may be dependent on GABA_B receptor subunit composition. Since the expression of many GABA_B receptor subunits are developmentally regulated, such dynamics should reveal advantage to advantage as to whether ethanol sensitivity is related to GABA_B receptor subunit makeup. Here, we combined patch clamp recording and RT-PCR in single immature Purkinje cells to link ethanol-GABA interaction with GABA_B receptor subunit mRNA expression, focusing on the long and short splice variants of the y2 subunit (y2L &y2S).

Purkinje cells cultured dissociated from postnatal day 1 (PD)-1, 3, 5, 7, 9 and 11 rat cerebellums, were used. Their dendritic fields were analyzed and 107-117 Purkinje cells were harvested. GABA-activated whole-cell current responses were potentiated upon co-application of 50 mM ethanol. Profiling of GABA_B receptor subunit mRNAs in these cells revealed the expression of a y2_1 and subunit mRNAs. The y2_1 subunit expressed prior to PD7 was that of y2S. The expression of y2L was not evident in individual Purkinje cells until PD7. In whole cerebellar tissue, y2S mRNA expression was found throughout postnatal development but that of y2L mRNA only beyond PD5. Other subunit messages also appear developmentally regulated.

Our results indicate that ethanol modulation of GABA_B receptor function in cerebellar Purkinje cells is present prior to the emergence of, and thus independent of, y2L mRNA expression and that the dynamics of neuroreceptor expression in development can provide insights into the specificity of ethanol-neuroreceptor interactions.

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THURSDAY AM
Prenatal Ethanol Exposure Alters Modulation of the GABA, Receptor Chloride Complex in Adult Offspring. D.R. Savage, J.L. Paxton, H. Wu, and A.M. Atlas*. Dept. of Pharmacology, Univ. of New Mexico H.S.C., Albuquerque, NM 87131-5316 The amino acid neurotransmitters glutamate and GABA have been implicated as neurochemical correlates underlying the electrophysiological and behavioral responses observed in Fetal Alcohol Syndrome. To further investigate prenatal ethanol-induced changes in the GABA system, we measured the ability of GABA and four modulatory agents that alter GABA-mediated receptor responses in hippocampal slices prepared from hippocampus, cerebellum and frontal cortex of adult offspring from ad lib chow, pair-fed and ethanol fed dams. Prenatal ethanol treatment involved the consumption of a liquid BioServ diet containing 5% (v/v) ethanol by the dams. Dose dependent GABA-stimulation of chloride flux was not significantly affected by the prenatal ethanol exposure in any of the brain areas examined. In the frontal cortex, prenatal ethanol exposure significantly attenuated both the positive modulatory effects of flunitrazepam (FLU 25 μM) and alphaxalone (ALPH: 25 μM) as well as the negative modulation by the inhibitory neurotransmitter 5-μg-precipitin 3-[3H]-25-μg-one sulfate (PB; 25 μM) and the benzodiazepine inverse agonist FG-7142 (FG; 10 μM) on GABA-stimulated 36Cl flux. In the cerebellum, prenatal ethanol exposure diminished the modulatory influences of the benzodiazepines but not the neurotransmitters. In hippocampus, prenatal ethanol exposure enhanced the effect of positive modulators (FLU and ALPH) while diminished the effects of negative modulators (PG and PB). These findings suggest that prenatal ethanol exposure produces long-lasting effects on the modulatory sites of the GABAergic receptor complex. Further studies will be required to determine whether these alterations are a compensatory mechanism in response to the early ethanol exposure or if these alterations contribute to the electrophysiological and behavioral deficits observed in fetal ethanol exposed offspring.

824.9

Effect of Prenatal Ethanol Exposure on Postnatal Expression of Glutamate Receptors and Physiological Responses in Adult Mice. N. E. R. Steiner, D. D. Davidson, and D. J. Marquiez-Orozco. Dept. of Pharmacology, Pharmacology, and Physiology, Univ. of New Mexico, Albuquerque, NM 87131-0001. Prenatal ethanol exposure leads to alterations in the expression of glutamate receptors in postnatal rat cortex. There is reduced expression of glutamate receptor subunits NR1, NR2A/B as detected by immunocytochemical analysis, which is maintained for at least 3 months after birth. However, we do not see a similar reduction in the expression of AMPA receptor subunits at the ages examined. Compared to normal animals the cortex of rats prenatally exposed to ethanol showed an increase in GluR1 immunostaining at P21 and P30, whereas GluR2/3 immunoreactivity was lower at P21 and higher by P30 compared to normal. The expression of GluR4 does not seem to be affected by prenatal ethanol exposure at P21 and P30. We are currently examining the effect of these subunit changes on physiologically recorded plasticity. Extracellular single unit recording suggests that neuronal responses in the primary somatosensory cortex of adult rats are altered following exposure to alcohol during gestation. The main differences compared to normal animal are in the level of spontaneous activity, the amplitude of evoked responses in the center and surround receptive fields and the response latencies. (Supported by NS 13031 and HD 1052)

824.10

Corpus Striatum Histological Alterations in Adult Mice Prenatally Treated with Diazepam A. M. M. Brown, J. M. Carter, and J. R. F. M. O'Sullivan. Dept. of Anatomy and Embryology, College of Medicine, University of Cincinnati, Cincinnati, OH 45221. Diazepam accumulates in the fetal human and mouse corpus striatum, where it produces a complex neurobehavioral and neurochemical differentiation. An atypical chromatin distribution and less number of fibers. We investigated if the histological alterations of the fetal corpus striatum occurs in adult mice. Single daily doses (2.7 mg/kg) of diazepam were administered to CD-1 strain female mice, from gestation day 6 to 17. A control received equivalent volumes of saline solution. The offspring's were wean-tasered by non-treated mice, and kept for 210 days. The motor activity and swimming activity were periodically measured from the 6th day until the 8th month. Mice were deeply anesthesized, perfused with 10% formaline, and the brains removed, fixed and stained for myelin, nerve cells, and axons identification; a selected blocks were impregnated with the fast-Golgi technique. Light microscopy demonstrated in diazepam mice atypical distribution of neurons, less number of fibers, and accumulation of glial cells around vessels, and in the bundles fibers. Behavioral analysis of the experimental group (swimming, and spontaneous motor activity) correlated with the persistent histological alterations.

824.11

Persisting Effects of Prenatal Exposure to Diazepam (DZP) on Sexually Dimorphic Reproductive Behaviors. M. M. Marquez-Orozco*, A. Martinez-Valero*, B. Victoria-Romero, A. M. M. Brown, and M. M. M. Brown. School of Research in Reproduction. School of Medicine, BUAP and Dept. of Embryology. School of Medicine, UNAM, Mexico 04510 D.F., Mexico. We have shown that drugs working on the sexual systems, effects of prenatal exposure to diazepam (DZP) on sexually dimorphic reproductive behaviors. In this work, we assessed the sexual behavior of smilie females CD-1 strain mice exposed to DZP during gestation. One group of female mice was exposed to DZP (2.5 mg/kg/day) from the 7th to the 17th days of gestation and a control group received saline solution. The 27th month of age, the spontaneous female sexual activity to males from the same breed was tested and video-recorded under red light. Premounting and copulating activities were evaluated. No difference was found in premounting behaviors from both groups, but during copulating stage lordotic indexes and proportion of lordotic females were greater in experimental animals. Results indicate persistent and long-lasting mating facility patterns of prenatal exposure of DZP on sexual behaviors which could be due to permanent modification of neurotransmission by the drug during development.

824.12

Histological Changes in the Retina of Mouse Fetuses Exposed to Diazepam. M. M. Brown, J. M. Carter, and J. R. F. M. O'Sullivan. College of Medicine, University of Cincinnati, Cincinnati, OH 45221. We investigated if diazepam (DZ) 1.0 mg/kg dose produces similar histological effects in the fetal retina as with 2.7 mg/kg dose. Three gestating CD-1 strain mice groups were injected daily sc from day 6 to 17, the first group with single daily DZ doses (2.7 mg/kg), the second group with single daily DZ, doses (1.0 mg/kg) and the third received saline solution. The fourth group was non-treated (NT). All were killed with CO2 atmosphere the 18th. day, and the fetus removed. Their eyes were fixed with 2.5% glutaraldehyde, post-fixed in OsO4, and embedded in epoxy resin. The semifeine sections were stained with toluidine blue and observed under the light microscope. The fetal retina in both DZ groups revealed in the neuroblastic layers delay cellular differentiation. A greater nucleus density of the retinal cells were observed. The cells showing nuclei with clumps of heterochromatin atypically distributed S and NT groups exhibited neither histological differences in the retina and the cells showing nuclei with clumps of heterochromatin atypically distributed. Results give evidence that both DZ doses produces histological changes in the fetal retina.
DRUGS OF ABUSE: ALCOHOL AND BENZODIAZEPINES
THURSDAY AM

282.14
THE EFFECT OF INTRAMYGLOCUDAL ADMINISTRATION OF FLUMAZENIL IN DIAZEPAM-DEPENDENT RATS. J.W. SLOAN*, F.P. WALK, X. HU, Dept. of Anesthesiology, College of Medicine, Univ. of KY, Lexington, KY 40536

The amygdala is an important brain site for the anxiolytic and anticonvulsant actions of benzodiazepines. Several pharmacological studies indicate a high density of BZ receptors (RZ) (predominantly type-2) in the basolateral amygdaloid complex. The aims of the present study were to test the hypothesis that chronic benzodiazepine administration (PC), fluvoxamine (FLU), to precipitate an abstinence syndrome in the ventromedial lateral (LaVM) and basolateral (BLA) amygdaloid nucleus in diazepam (DZ)- dependent rats. The rats were chronically treated for 14 days with PC (5 mg/kg) or FLU (5 mg/kg), slowly released from subchronically implanted capsules. FLU (25 μg) and DMSO vehicle were microinjected into LaVM (AP=±5.7; RL=4.8; Y=-3.1±1.8). The abstinence signs, behavioral activities and EEG were recorded for 10 min before and 40 min after microinjections. The Precipitated Abstinence Score (PAS), Behavioral Score (BS) and the changes in Total Power (TP) of the EEG recorded at the sites of microinjections were determined. FLU did not produce either a significant PAS or a significant BS in BLA and LaVM. FLU evoked chronic convulsions (1 rat) and twitching and jerks (P=0.05 in BLA. No signs of abstinence were precipitated by FLU in LaVM. FLU tended to increase TP of low and high frequency bands in BLA but not in LaVM. DMSO did not affect the BS and LaVM. The present results indicate that except for convulsant signs a surprisingly mild abstinence was evoked by FLU in both nuclei of lateral amygdala in rats dependent on PC (supported by NIDA grant DA13195).

282.15
INTRATHALIC (IT) ADMINISTRATION OF FLUMAZENIL AND PK11195 PRODUCE AN ABSTINENCE SYNDROME IN DIAZEPAM-DEPENDENT RATS. E.P. WALK, J.W. SLOAN, X. JING, P.H. HOLMAN, Dept. of Anesthesiology, College of Medicine, Univ. of KY, Lexington, KY 40536.

The actions of central and peripheral benzodiazepine receptor (BZR) antagonists, flumazenil (FLU) and PK11195 (PK), on the spindle level were determined in rats subchronically implanted for 3 wks with silastic capsules with dissolved PK (540 μg). Control rats were exposed to empty capsules. The rats were implanted with EEG electrodes into the ventral thalamic nucleus (TH), CA1 area of hippocampus (HP), and parietal cortex (PC) and cerebellum (CC) cortices and with an intrathalamic (IT) catheter into the spinal subarchnoid space (vxicity of T-12). After recovery, FLU (0.1 mg/kg), PK (0.65 mg/kg) and vehicle were injected (i.p.) IT. The EEG, abstinence signs and behavioral activities were recorded. The Precipitated Abstinence Score (PAS), Behavioral Score (BS) and changes in Total Power (TP) for (d=0.13) and fast (f=18-24Hz) IT frequencies were determined. Intrathalamic FLU and PK precipitated a significant PAS but not a significant BS in DZ and baseline (BLA) rats. Intrathalamic PK decreased TP of fast waves in TH, PC, and CC but not in IT. FLU did not affect TP. In control rats PAS, BS and changes in TP were not significant, thus, in the spinal cord both central (predominantly type-2) and peripheral BZR are involved in dependence on DZ as indicated by the PAS produced by FLU and PK. The lack of effect of IT FLU on EEG may be related to the low density of type-1 BZR in the spinal cord. (NIDA grant DA13195).

282.17
EFFECTS OF DIAZEPAM ON GABA B RECEPTOR FUNCTION IN THE TOLERANT AND WITHDRAWAL RATS. S. TAKI, T. SATO*, A. NAKASHIMA, H. TSUJI, Y. MASIYAMA, N. TAKAHASHI, Department of Neuropsychiatry and Pharmacology, School of Medicine, Sapporo University Medical School, Sapporo, 060 Japan

Alterations in GABAB receptor function were studied in diazepam (DZP)-dependent rats. Physical dependence on DZP was induced in male Fischer rats by the drug-admixed food (DAF) method. Using the DAF method, we examined differences in the physical dependence on DZP characterized by spontaneous convulsions during DZP withdrawal developed. In comparison with control rats, 10mM GABA-dependent 35Cl-influx in withdrawal rats were significantly increased, whereas there was no significant difference between the tolerant and control rats. While enhancement of GABA-dependent 35Cl-influx by the addition of BZ and Flumazenil (FLU) to the medium was observed in the tolerant rats, there was no such effect of BZ or FLU in the DZP-tolerant animals. On the other hand, GABA-dependent 35Cl-influx was enhanced by the BZ in the withdrawal group. The BZ-induced binding may be present in the GABAB receptor, Bmax values were significantly increased in DZP-withdrawn animals, but decreased in the DZP tolerant group as compared with the control. The present results indicate that GABAergic transmission involving the regulation of GABA-dependent chloride channels is altered in DZP-dependent rats.

282.18
GENDER-SPECIFIC CHANGES IN CRF CONTENT AND CORTICOSTERONE LEVELS FOLLOWING CHRONIC BENZODIAZEPINE EXPOSURE IN RATS. M.A. WINTER* and R. BISCUITI, Department of Pharmacology, Univ. South Carolina School of Medicine, Columbia, SC 29208.

Acute stress induces sex-specific changes in GABA/benzodiazepine (BZ) receptors and corticosterone release in rats. Gender-related differences have also been observed in GABA/BZ responses after chronic benzodiazepine exposure. The present study compared changes in CRF content and corticosterone release following benzodiazepine exposure in ovariectomized female (O VX) and sham-operated male rats. After treatment with vehicle or diazepam-filled (DZ) silastic capsules for 3 days (acute) or 3 weeks (chronic), CRF content in punches from eight brain areas and serum corticosterone were determined in female and male rats (all treated with hypothalamic to sacrifice procedure) and swim-stressed (10 min prior to sacrifice) rats. In OVX females, acute and chronic DZ exposure attenuated the stress-induced increase in corticosterone by 31% and 32%, respectively (P<0.0001). In male rats, DZ exposure significantly decreased in corticosterone levels (15%) after acute and chronic DZ exposure. Chronic diazepam exposure significantly increased CRF content in amygdala of stressed OVX females, but not males (F=8.2, P=0.01 for sex-difference interaction). Median esteem levels of CRF were reduced by chronic DZ treatment in stressed males, but not in OVX females (F=5.6, P=0.02 for interaction). Several brain areas (proprectic area, locus coeruleus, paraventricular nucleus) showed male-OVX differences in CRF levels, but no effect of DZ exposure. CRF content in some areas was not altered by gender or DZ treatments. The results suggest BZ agonists attenuate stress-induced corticosterone release to a greater degree in females than males, but that tolerance does not develop to this effect. Chronic benzodiazepine exposure appears to modulate CRF content of certain brain areas in a gender-specific manner. (Support: DA03932 to MAW).
DRUGS OF ABUSE: ALCOHOL VI

824.10


The discriminative stimulus effects of partial agonists at the Bz site were studied in rats trained to discriminate Bz or pentobarbital. In a two-lever drug versus no drug procedure, bremazolam, midazolam, and U-78875 reliably occasioned drug-appropriate responding in animals trained to discriminate diazepam and pentobarbital but there was less reliable generalization in animals trained to discriminate lorazepam. In rats trained to discriminate two doses of midazolam under a three-lever procedure, bremazolam occurred 19% of drug-appropriate responding only on the lever paired with the 0.32 mg/kg dose but not the 3.2 mg/kg dose. In rats trained to discriminate lorazepam from pentobarbital under a three-lever procedure, midazolam occasioned responding on both drug-paired levers; although, across the dose range, responding was higher on the lorazepam lever for rats trained to discriminate lorazepam first and pentobarbital second. In interaction studies in rats, U-78875 antagonized the effects of lorazepam, had little effect on diazepam, and potentiated the effects of pentobarbital. There appeared to be a behavioral component to the ability of U-78875 and bremazolam to antagonize lorazepam's effects in baboons: the compound antagonized lorazepam when the baboon did not generalize to it, but potentiated lorazepam in the partial agonist shared discriminative effects with lorazepam. Supported in part by NIDA DA04133.

824.20

ALPRAZOLAM DEPENDENCE PREVENTED BY SUBSTITUTING WITH THE 8-CARBOLINE ABERCARNIL, G. Pinna, R. Galici, H.H. Schneider, D.N. Stephens* and L. Teirst, Research Laboratories of Schering AG, D-13342 Berlin, Germany.

Abrupt termination of the treatment of humans with benzodiazepines (BDZs) leads to a rapid-onset discontinuation syndrome. For this reason, there is considerable interest in discovering BDZs with reduced risk of discontinuation syndrome. We have employed methods for electroencephalographic (EEG) monitoring of seizures, for electromyographic monitoring of muscle tone, and for detecting anxiety-like behavioral changes after discontinuation of BDZ treatment with sedative drugs in mice for controlled and standardized assessment of dependence liability of alprazolam. Alprazolam, a BDZ with a short half-life, was chosen because it has replaced diazepam as the most prescribed anxiolytic drug since the end of eighties. Male NMRI mice, 20-24 g, were subjected to s.c. injections of 6 mg/kg of alprazolam or vehicle given twice daily for 12 days. Long-term treatment with alprazolam led to a rapid loss of its depressant action on exploratory activity in non-habituated mice. Monitoring of withdrawal signs started on the day following the last administration of alprazolam or vehicle. The intensity of the discontinuation syndrome (EEG seizures, rigidity, anxiety) increased slightly over first 2.6 days of withdrawal, the symptoms were most pronounced during the next 7-14 days, and abated slowly up to withdrawal day 21-28. Replacement of alprazolam treatment with the BDZ 8-carboline selective agonist abercarnil (6 mg/kg for 7 days) prevented the occurrence of withdrawal signs; when replacement treatment with abercarnil was subsequently terminated no signs of dependence were detected. Replacement of alprazolam treatment with the 8-carboline antagonist ZK93426 (ethyl-5-isoxazopyro-4-methyl-6-carboline-3-carboxylate; 20 mg/kg for 7 days) did not prevent the discontinuation syndrome. Replacement therapy with abercarnil after long-term treatment with the BDZs offers novel method for rapid tapering. The use of the BDZ antagonists for tapering long-term treatment with BDZs is not justified by the experimental data.
885.3

CHARACTERIZATION OF GLUTAMIC ACID DECARBOXYLASE PROTEIN FUNCTION AND A mRNA CONTENT IN GENETIC MODELS OF SEVERE OR MILD WITHDRAWAL NEUROEXCITABILITY, AND D. Wu. Department of Medical Psychology, Oregon Health Sciences University, Portland, OR 97201-8998, USA.

Physical dependence and withdrawal from alcohol and other central depressants shows the influence of heritable factors, but none of the genes responsible has been identified. By association and interval mapping, we identified a quantitative trait locus (QTL) which accounts for more than 40% of the variance in ethanol withdrawal neuroexcitability in C57Bl/6J (B6) X DBA/2J (D2) recombinant inbred (BIX RI) strains. Analyses using a BIDF2 intercross confirmed that a QTL affecting withdrawal severity is linked to the microsatellite marker D2Mit111, which localizes to the same region of chromosome 2 as Gad-1 (37-43.8). Gad-1 encodes glutamic acid decarboxylase (GAD), which catalyzes the rate limiting step in the synthesis of the major known inhibitory neurotransmitter GABA (GABA). Numerous studies have implicated the synaptic actions of GABA in neuroadaptation to ethanol associated with physical dependence and withdrawal.

DBA/2J mice are a well characterized genetic animal model expressing severe ethanol dependence and withdrawal neuroexcitability, whereas B6 have mild withdrawal reactions. We found that D2 mice demonstrate lower GAD enzyme activity as compared to B6 mice. Northern blot analyses using RNA isolated from ethanol-naive and ethanol-dependent B6 and D2 mice show that GAD mRNA content (3.7 kb and 5.7 kb isoforms, encoded by Gad-1 and Gad-2, respectively) is regulated by chronic ethanol treatment. These results indicate that genetic differences in ethanol withdrawal neuroexcitability may be mediated, in part, by differences in GAD expression and function. (Supported by NIH grants R01 AA06243, P01 AA06821, and T32AA07608)

885.5

CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL SELECTIVELY INCREASE DIAZEPAM-INSENSITIVE [3H]RO5 14-513 BINDING IN MOURE CEREBELLUM. H.C. Beeler* and M.F. Lewis. Med. Univ. of South Carolina and VAMC, Charleston, SC 29425 and Rhone-Poulenc Rorer Central Research; Collegeville, PA.

The partial benzodiazepine inverse agonist, RO 14-5135 has been shown to block some behavioral and biochemical effects of ethanol (EtOH) ([3H]RO 14-5135 inhibits the classes of recognition sites in the mammalian cerebellum, a diazepam-sensitive (DZ-S) site and a diazepam-insensitive (DZ-IS) site that may represent a novel subtype of the GABAA receptor. The present studies were conducted to examine the effects of chronic ethanol exposure on both total and DZ-IS ([3H]RO 14-5135 binding in cerebellum from C57B16 mice. Male mice received either 64 h EtOH intoxication via inhalation chambers (BEC = 185-186 mg/dl) or no EtOH exposure. Mice were sacrificed at the end of the EtOH exposure (H 6) or 8 hours post withdrawal (8P). Ligand binding studies conducted (125 I) were conducted on pooled cerebellar tissue from 3-4 mice. In control mice, the binding parameters for [3H]RO 14-5135 were Kd = 3.9 ± 1.1 nM, Bmax = 320 ± 300 fmol/mg protein. The binding parameters for [3H]RO 14-5135 in the presence of 10 μM DZ (DZ-IS) were Kd = 3.0 ± 0.4 nM, Bmax = 953 ± 108 fmol/mg protein. Neither EtOH intoxication (HR) nor EtOH withdrawal (HR 8) significantly altered total ([3H]RO 14-5135 binding density in the mouse cerebellum. However, DZ-IS ([3H]RO 14-5135 binding density was significantly increased (80%) by EtOH intoxication and by (75%) EtOH withdrawal. These results suggest that chronic ethanol exposure and withdrawal selectively increase DZ-IS ([3H]RO 14-5135 binding sites in mouse cerebellum. These data agree with other studies indicating an increase in mRNA and protein levels for the δ subunit, which encodes DZ-IS RO 14-5135 binding at GABAA receptors in cerebellum.

885.7

THE IMIDAZOBENZODIAZEPINE INVERSE AGONIST RO19-4603 (RO19) ATTENUATES ETHANOL (EtOH) ORAL SELF-ADMINISTRATION (OSA) IN SPEARLING-DAWLEY RATS. G. G. Blakley*, M. P. Peterson, H. L. June, and M. J. Lewis Neurobehavioral Laboratory, Dept. of Psychology, Temple University, Philadelphia, PA 19122 and IUPUI, Indianapolis, IN 46202

Benzodiazepines (BDZ) are known to have antinociceptive actions. BDZ-agonists have been reported to antagonize several of EtOH actions including its reinforcing properties. RO19 is a potent imidazobenzodiazepine inverse agonist. Although having a half-life of 15-60 min, a single dose of RO19 has been reported to suppress EtOH-induced OSAs in alcohol preferring (P) and non-prefering (NP) rats for as long as 32 hrs. The present experiment examined the effect of RO19 upon OSA of EtOH in rats that trained for free-choice, drug-induced Sprague-Dawley rats. Animals were given daily, 1 hr concurrent access to H2O and gradually increasing EtOH concentrations (1%-10%) (v/v). After stable preference of a 10% solution was established, animals were pretreated with i.p. injections of 0.06g/kg, 0.1g/kg, or 0.15g/kg of RO19. RO19 showed a dose-dependent attenuation of EtOH consumption over several hours. These data are consistent with previous research on RO19 in selected rats and suggest that brief occupation of GABA/BDZ receptor sites by such compounds may alter EtOH reinforcement. (Supported in part by AA0263 and RR08016 and Temple University.)

885.8

ETHANOL SUBSTITUTES FULLY FOR A DIAZEPAM-ETOHAMINE MIXTURE. J. Jenkins, Y. Eplimter, B. Rocha and M. Emmett-Olasyik. Dept Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107

When ethanol (EtOH) is trained as a discriminative stimulus, drugs that enhance GABAergic neurotransmission (e.g., diazepam, DZP), and drugs that uncompetitively antagonize glutamate neurotransmission at NMDA receptors (e.g., ketamine, KET), substitute for EtOH. However, when drugs from either of these classes are trained as discriminative stimuli, EtOH fails to substitute fully for either type of drug. The present study tested the hypothesis that EtOH can be trained to discriminate a mixture of EtOH (5% mg/kg) and KET (10 mg/kg) from saline. EtOH would substitute fully for this training mixture. The mixture was trained using a two- lever choice procedure in which food served as a reinforcer under a fixed-ratio 10 schedule. After the discrimination was acquired, dose-effect testing showed full substitution with: the mixture, DZP alone, KET alone, pentobarbital, chloralazepoxide, diazepam and EtOH. The mixture no longer supported the hypothesis that activation of GABAergic neurotransmission and blockade of glutamate neurotransmission are critical in producing an EtOH life stimulus. Supported by R01 AA9378.
825.10

THE EFFECTS OF LOW LEVEL HYPERBARIc EXPOSURE ON 4,5,6,7-
TETRAYDROXOXYL-PROVIN-3-OL (THP), PENTOBARBITAL- AND
ETHANOL-INDUCED DURABILITY IN RATS.
Althet, and R.L. Balog. Department of Pharmacology and Toxicology, School of
Pharmacy, University of Southern California, Los Angeles, CA 90033.
Exposure to 12 atmospheres absolute (ATA) helium oxygen gas (heliox)
aglulizes behavioral effects of ethanol, n-propanol and diazepam, but
not morphine, nicotine, or isoflurane. This work recently demonstrated
that exposure to 12 ATA heliox antioxidanted ethanol-potential
of GABA-activated CI uptake in mouse cerebrocortical membrane vesicles
(microsomes) without altering GABA-stimulation per se. The pattern of
the antagonist suggests that alcohol acts through a selective pattern
of drugs that act through alloglutamatergic modulation. The present study
further investigates the selectively of low level hyperbaric exposure. C57BL/6J mice
were injected i.p. with 50 mg/kg pentobarbital (GABA receptor antagonist) or 3.6 g/kg ethanol. When
LORR occurred, the mice were placed in a modified rat-exposure chambers
and exposed to either 12 ATA heliox or to control atmospheric
conditions of 1 ATA air (O2:21%). The mice at ambient temperature
exposed to offset-drug and helium-induced hypothermia. Exposure to 12 ATA heliox
significantly reduced LORR duration induced by pentobarbital and ethanol but
did not significantly reduce LORR duration induced by THP. These results add to
the evidence that low level hyperbaric exposure selectively antagonizes
the effects of drugs which act through alloglutamatergic modulation of GABA,
receptors, (e.g., ethanol, diazepam and pentobarbital) but does not antagonize
the effects of drugs that act via high affinity binding as direct
channel blockers (e.g., GABA, picrotoxin and morpina) and suggest that
alloglutamatergic modulation in alloglutamatergic regions
may be involved in alcohol-induced hypothermia.
Supported by NIAAA grants AA09797 and AA05246.

825.11

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF hIMKD01 BINDING
TO NMDA RECEPTORS IN BRAINS OF RODENTS DIFFERENTIALLY
SENSITIVE TO N-METHYL-D-ASPARTATE (NMDA) SELF-
ADMINISTRATION. R.J. Selfcot* and R.L. Balog. Department of
Pharmacology and Toxicology, Medical College of Virginia,
Richmond, VA 23296-0101.
Animal studies suggest that the nucleus accumbens (NAC) is a brain
area involved in the rewarding effects of ethanol (E). Recently we
reported that low BtOH concentrations decreased NMDA-induced currents in
NAC core neurons in neonatal rats (JPH, 1999). Overall, these
evidence suggest that chronic ETOH treatment alters NMDA
function. To determine whether such changes are necessary for
antagonist-sensitive sites in the NAC. We used voltage-clamp recording in rats
nucleus accumbens slice preparation to compare the NMDA receptor sensitivity of NAC core
eurons taken either from rats maintained in ethanol for 3-4
weeks and then withdrawn for 8-12 hours (ETOH treated; mean BAL: 71-
138 mg/dl) or from rats killed at the same period in
controls without ethanol vapor (controls). We rapidly superfused NMDA for 3 min, in the
presence of 10 µM CNQX, 1 µM tetrodotoxin and 30 µM bicuculline
to prevent indirect or non-NMDA-mediated effects. Currents produced by
NMDA superfusion were measured over a period of 0.5-180 s. We observed no differences in
transient AMPA currents or in the kinetics of desensitization in both
NMDA receptor-sensitive and -insensitive NAC core neurons.
NMDA-induced currents and peak amplitudes were not different between
NMDA receptor-sensitive and -insensitive NAC core neurons. NMDA-induced
currents were significantly reduced in NMDA receptor-sensitive NAC core
neurons taken from rats maintained in ethanol for 3-4 weeks and then withdrawn for 8-12 hours.

825.12

WITHDRAWAL FROM CHRONIC ETHANOL EXPOSURE INCREASES
SENSITIVITY TO N-METHYL-D-ASPARTATE (NMDA) IN RAT NUCLEUS
Department of Neuropharmacology and Alcohol Research Center, The Scripps Research Institute, La Jolla, CA 92037.
Behavioral studies suggest that the nucleus accumbens (NAC) is a brain
area involved in the rewarding effects of ethanol (E). Recently we
reported that low BtOH concentrations decreased NMDA-induced currents in
NAC core neurons in neonatal rats (JPH, 1999). Overall, these
evidence suggest that chronic ETOH treatment alters NMDA
function. To determine whether such changes are necessary for
antagonist-sensitive sites in the NAC. We used voltage-clamp recording in rats
nucleus accumbens slice preparation to compare the NMDA receptor sensitivity of NAC core
eurons taken either from rats maintained in ethanol for 3-4
weeks and then withdrawn for 8-12 hours (ETOH treated; mean BAL: 71-
138 mg/dl) or from rats killed at the same period in
controls without ethanol vapor (controls). We rapidly superfused NMDA for 3 min, in the
presence of 10 µM CNQX, 1 µM tetrodotoxin and 30 µM bicuculline
to prevent indirect or non-NMDA-mediated effects. Currents produced by
NMDA superfusion were measured over a period of 0.5-180 s. We observed no differences in
transient AMPA currents or in the kinetics of desensitization in both
NMDA receptor-sensitive and -insensitive NAC core neurons. NMDA-induced
currents were significantly reduced in NMDA receptor-sensitive NAC core
neurons taken from rats maintained in ethanol for 3-4 weeks and then withdrawn for 8-12 hours.

825.13

THE ANTI-CRAVING AGENT ACAMPROSATE ENHANCES NMDA-
MEDIANATED EPSPS IN RAT NUCLEUS ACCUMBENS NEURONS.
F. Bettin, W. Francesch, S.G. Madamba, W. Ziegler, and D. R. Sigurd*,
Department of Neuropharmacology and Alcohol Research Center,
Research Institute of Scripps Clinic, La Jolla, CA 92037, University of
Pisa, Italy, and Clinical Inst., Max-Planck-Institute of Psychiatry,
D-80804 Munich, Germany.
Acamprosate (N-acetylcysteine prodrug; LPHFA) is a new drug shown in
European clinics to prevent relapse in waneen alcoholics. However,
the mechanisms of this action are unclear. Therefore, we studied the effects of acamprosate
in a slice preparation of nucleus accumbens (NAC), a brain region thought to play a role in drug reinforcement.
Recent studies in our lab (271, 1566, 1994) demonstrated
that ethanol (EtoH) inhibits glutamatergic NMDA- and
non-NMDA-mediated currents in the NAC. We measured currents and voltages
clamp recordings of NAC core neurons and isolated locally-evoked
NMDA and non-NMDA glutamatergic EPSP components with 20 µM CNQX
and 50-60 µM AMP, respectively. Bicuculline 30 µM was also present to block
GABA receptors. We recorded from 20 neurons with a mean resting membrane
dm of 65.8 ± 3.2 mV and mean input resistance of 40 MΩ. Superfusion of 300 µM acamprosate did not
alter Ringer's solution input resistance in these neurons. However,
acomprosate significantly increased NMDA EPSPs and EPSPs in 70% of
neurons tested (n = 10; F(1,30) = 7.646; p = 0.0096; neurons showed no
effect). In 11 neurons, the input resistance was significantly increased.
These results are compatible with previous findings in hippocampus (Madamba et al.,
in submission) and in cortex (Zezze et al., Eur J. Pharmacol. 231: 47, 1993)
suggesting that the clinical efficacy of acamprosate may result from modulation of glutamatergic neurotransmission.
Supported by grants from NIH (AA04920) and Groupe LPHFA (Lyon, France).
825.15

**DISCRIMINATIVE STIMULUS EFFECTS OF THE NOVEL NEUROTENSINAGONIST CO 8-7071, IN PENTOBARBITAL-TRAINED RHESUS MONKEYS. J.K. Rookel* and W.L. Woolworth. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216.**

Co 8-7071 (3x,15-dihydroxy-5-3 trifluoromethyl-5-pregn-20-one, 21-hemisuccinate) is a water-soluble, orally bioavailable neurosteroid pro-drug. The purpose of this study was to assess the discriminative stimulus (DS) effects of CO 8-7071 in rhesus monkeys (N=7) trained to discriminate pentobarbital (PB, 10 mg/kg, i.p., 60 min pre-session) from saline. Lever pressing was maintained under a discrete-trials shock avoidance schedule of reinforcement (30 trials/day, 30-sec ITI, FR 5). During test sessions, in which responding on either lever was reinforced, the monkeys were given PB (3.0-17 mg/kg, i.p., 60 min pre-session) or saline. Co 8-7071 (3.0-30 mg/kg, i.g., either 60 or 120 min pre-session). Administration of PB resulted in a dose-related increase (10-100%) in the percentage of responses emitted on the drug-appropriate lever with a dose-related decrease in response rate (rise). Co 8-7071 produced drug-appropriate responding in both monkeys, depending on pre-session time. At 60 min pre-session, Co 8-7071 (3.0-17 mg/kg) produced a dose-related increase (10-100%) in drug-appropriate responding in one monkey, but not in the second monkey. At 120 min pre-session, Co 8-7071 (3.0-30 mg/kg) produced a dose-related increase (10-100%) in drug-appropriate responding in the one monkey tested to date. No systematic effects on response rate were seen after Co 8-7071 treatment. These results suggest that Co 8-7071 may share some DS effects with PB in rhesus monkeys. (Supported by NIDA grant DA-09139).

825.16

**BEHAVIORAL PHARMACOLOGY OF NEGATIVELY-PUNISHED RESPONDING IN THE RAT. J.M. Kilduff, J.A. Esh, and J. Breese. Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814.**

Behavior may be suppressed by both positive and negative punishment. Positive punishment consists of presentation of a novel, appetitive stimulus, whereas negative punishment consists of the removal of an appetitive stimulus. These effects were studied in the rat. Conditions of negative punishment included: (1) continuous avoidance of aversive events such as shock. In contrast, negative punishment suppresses behavior by response-contingent removal of access to positive reinforcement (timeout). The effects of CNS drugs on negatively-punished responding with emphasis on identifying similarities and differences in the effects of drugs on responding punished by negative versus positive punishment. Initially the lever-pressing of 4 rats was maintained by a fixed-interval (FI) schedule in which the 1st response after 1 min produced a food pellet. Negative punishment was subsequently introduced so that only 1 response produced a timeout during which the response lever was retracted and reinforced, and the 1-min (fixed-interval) timer was paused. After 1 min the lever was re-extended into the chamber, and timing resumed. Introduction of punishment decreased the response rate from 623 responses/sec to 168 responses/sec. After punishment had stabilized, drugs were administered before some sessions. The following drugs increased punished responding: the psychomotor stimulant d-amphetamine; the sigma and PCP receptor agonist MK-801 (dizocilpine); the benzodiazepine chlordiazepoxide. The opiate receptor, the barbiturate pentobarbital, and the 5-HT1A receptor agonist 8-OH-DPAT failed to reliably increase rates. Results resemble those typically obtained with positive punishment in that responding in both procedures is increased by chlordiazepoxide and MK-801, and 8-OH-DPAT and morphine fail to increase rates. However, effects differ in that pentobarbital increases only positively-punished responding, and d-amphetamine increases only negatively punished responding. The results suggest distinct pharmacological profiles for the two forms of punishment despite similarity in their behavioral effects.

825.17

**EFFECTS OF CHLORDIAZEPoxide AND BUSPIrones ON BEHAVIOR SUPPRESSED BY PRESENTATION OF TIMEOUT FROM FOOD. Evan Haaren* and K.G. Anderson, Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611-2250.**

Six rats were exposed to a two-component multiple schedule. In one component (A), lever presses were followed by pellet presentation on a RI 30-s schedule, in the other component (B), pellet presentation occurred on the same schedule, but lever presses also resulted in timeout presentation (10 sec) on a RI 2-s schedule (comprised RI 30-s food, RI 2-s timeout). The two components were presented in an A-B-A-B-A sequence. Once responding had stabilized subjects were injected with different doses of chlordiazepoxide (CDP, 1, 3, 10, 17 and 30 mg/kg, IP, -15 min), or different doses of buspirones (BUSP, 0.1, 0.3, 1.0, 1.7, 3.0 or 4.2 mg/kg, IP, -15 min). Response rates during the concurrent component of the schedule were considerably lower than those in the other component. Low doses of CDP and BUSP produced a small increase in RI responding. Suppressed responding was greatly increased by low doses of CDP, but hardly affected, or decreased by low doses of BUSP. Higher doses of CDP ( = 10 mg/kg) and BUSP ( = 1.0 mg/kg) suppressed responding in both components of the multiple schedule.

826.1

**EFFECTS OF SYSTEMIC AND INTRACRANIAL CAFFEINE ON Dopamine OVERFLOW IN Rats. Madeleine L. RCaserta and John A. Leonard. University of Virginia, Charlottesville.**

The neural basis of caffeine's psychomotor stimulant actions is not completely understood. The possibility has been raised that as with other psychomotor stimulants (e.g. nicotine, amphetamine, and cocaine), the effects of caffeine are in part due to the activation of dopaminergic substrates. We studied the effects of caffeine on extracellular levels of dopamine (DA) in the nucleus accumbens of either anesthetized or freely-moving rats. Dyalysate samples were collected at 20-min intervals and DA levels were quantified using HPLC with electrochemical detection. Caffeine was administered systemically (0, 20 or 40 mg/kg, i.p.) or directly into the nAcc (0, 10, 100, 1000 µM). In anesthetized rats, systemic administrations of 40 mg/kg, but not 20 mg/kg, of caffeine increased DA overflow in the nAcc. In unanesthetized rats, however, both doses increased locomotor activity while having no effect on nAcc DA. When administered directly into the nAcc at 1000 µM, produced DA overflow in unanesthetized as well as anesthetized rats. In the former case, caffeine also had a locomotor stimulant effect. Even though caffeine can facilitate DA overflow in the nAcc under certain conditions (at high concentrations or under anesthesia) the relevance of such effects to its behavioral actions is not apparent. Because systemic injections of caffeine (20 & 40 mg/kg) do not alter DA overflow, it seems that caffeine's behavioral effects are not dependent on the facilitation of DA overflow in the nAcc.

826.2

**NMDA-TYPE GLUTAMATE RECEPTOR INVOLVEMENT IN CAFFEINE'S LOCOMOTOR STIMULANT EFFECTS AND TOLERANCE TO THESE EFFECTS. K.R. Powell* and S.G. Hoffman. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.**

The involvement of NMDA-type glutamate receptors in caffeine's locomotor stimulant effects and the development of tolerance to these effects was examined. Caffeine, administered i.p., increased locomotor activity in mice and rats. When combined with i.c.v. d,l-diazocilpine, the highest dose of dizocilpine, that alone did not increase locomotor activity (0.1 mg/kg), enhanced the locomotor stimulant effects of caffeine. Lower doses of dizocilpine (0.01-0.03 mg/kg) did not alter the effects of caffeine. During the tolerance phase of the study, rats were exposed to caffeine using scheduled access to a caffeine-infused drinking solution (1.0 mg/ml) and were implanted with osmotic minipumps (Alzet Corp.) containing either dizocilpine (0.1 mg/kg/day infusion) or saline for a period of 7 days. The effects of caffeine on locomotor activity were examined before, during and following chronic exposure to caffeine to measure the development of tolerance to these effects. Diazocilpine did not alter the development of tolerance to caffeine's effects. These data indicate a supradrug interaction between the acute effects of caffeine and dizocilpine and suggest that NMDA-type glutamate receptors may be involved in the mechanisms underlying locomotor stimulant effects of caffeine. On the other hand, these data suggest that NMDA-type glutamate receptors are not involved in the development of tolerance to caffeine's locomotor stimulant effects in rats. (Supported by DA 03413)
286.3 VENTRAL PALLIDAL 6-OHDA LESION IMPAIR AMPHETAMINE SELF ADMINISTRATION AND LOCOMOTOR ACTIVITY. N.J. DeCicco, L.M. Nishimura, and J.W. FFlexner. Dept. of Psychiatry, Univ. of Toronto, Toronto, Canada, MSS 1AT; and Clarke Inst. Psychiatry, Toronto, Canada, M5T 1R8.

Recent studies have suggested a role for the ventral pallidum (VP) in drug self-administration and locomotor activity. We examined the possible contribution to these behaviors of ascending mesopallidal dopamine (DA) neurons has not yet been evaluated. The present experiments sought to examine the role of VP DA in amphetamine (AMP) self-administration (SA) and locomotion in rats.

Rats were trained intravenously self-administer AMP (50 μg/injection) on a FR1 schedule (third day) until response rates stabilized. Rats were then treated bilaterally with 6-OHDA (6-OHDA, 2.4 μg/3.0 μl) or an acidified vehicle. Immunohistochemical results from pilot studies have indicated reliable VP DA depletions without affecting DA content in the ventral tegmental area, frontal cortex, or striatum. However, in exp. 1, following a 1 week recovery period, animals were again given access to AMP (50 μg/injection) and allowed daily 1 hr SA sessions for 10 days. In exp. 2, an AMP (100, 50, 25, 12.5, 6.125, and 0 μg/injection) dose response analysis was conducted over the subsequent 6 days. Finally, rats were tested for the expression of AMP-induced (1.0 mg/kg ip) locomotor activity in photo-behavior based motor activities in exp. 3.

Results: rats treated with 6-OHDA administered significantly fewer amphetamine infusions than control animals over the last 5 days of testing. Further, results from exp. 2 revealed that 6-OHDA treated rats displayed a decreased inverted-U doseresponse curve, administering significantly fewer infusions across middle range doses. In exp. 3, results indicated that animals treated with 6-OHDA were significantly less responsive to the locomotor activating effects of AMP. These results suggest the involvement of VP DA in the rewarding and locomotor activating effects of psychostimulant drugs.

286.4 CONDITIONED INCREASES IN MOTOR ACTIVITY AND DOPAMINE CONCENTRATIONS IN THE NUCLEUS ACCUMBENS OF THE RAT FOLLOWING REPEATED ADMINISTRATION OF COCAINE OR 4-AMPHETAMINE. S. Blaha and A.G. Phillips. Department of Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

One of the terminal regions of the mesocorticolimbic dopamine (DA) pathway, the nucleus accumbens (N.Acc.), has been implicated in the primary and secondary reinforcing properties of drugs of abuse. This study demonstrates that repeated pairings of either cocaine or 4-amphetamine (m-AMP) with a secondary non-pharmacological environmental stimulus (odor, light) produced conditioned increases in both locomotor activity and extracellular DA concentrations in the N.Acc., when rats were exposed to the compound CS in the absence of the drug. The CS was used to monitor extracellular levels of DA in the N.Acc. Motor activity was recorded as the number of photocell beam crosses in the test chambers. Compared to controls, both cocaine and m-amp significantly increased motor activity (mean maximum: 80 and 470 photocell counts, respectively) in the first 10 min after drug administration. Significant increases were also seen in associated DA oxidation currents that plateaued at 2NaA and 3.75NaA after cocaine and amphetamine, respectively.

When compared to controls, rats that had previously received either cocaine or m-amphetamine paired with the CS showed significantly increases in motor activity, upon presentation of the compound CS, that were of comparable magnitude to those seen in the animals treated with either drug. Associated changes in extracellular DA concentrations in the N.Acc. were significantly elevated ~20 min after extinguishment of the CS, to levels between 2-3NaA and 3.75NaA for the cocaine and amphetamine respectively. The present experiments provide support for the hypothesis that DA in the N.Acc. is involved in the conditioned incentive effects of drugs of abuse.

286.5 DOSE-DEPENDENT EFFECT OF METHAMPHETAMINE ON NEUROTENSIN RELEASE IN STRIATUM AND NUCLEUS ACCUMBENS. J.D. Walbridge, J.W. FFlexner, J.N. Doyle, and O.R. Hamdou. Dept. of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT 84112.

Central neurotransmitters (NT) are thought to play an important role in the modulation of dopaminergic pathways. Stimulants of abuse such as methamphetamine (METH) dramatically alter tissue levels and synthesis of NTs. However, the regulation of NT release has been difficult to study in vivo as extracellular levels of NT are extremely low. We previously measured extracellular dopamine (D1 and D2 receptors) in microdialysis in awake animals, and demonstrated regulation by D-2 receptors. The present study examines the effect of METH on NT release from striatum and nucleus accumbens, and correlates those changes with the expression of dopamine D1 and D-2 receptors in mediating METH-induced alterations. METH alters extracellular NT in a dose-dependent manner. At a low dose (0.5 mg/kg), METH had no significant effect on NT release. The role of DA receptor subtypes was assessed in the striatum by combining selective D-1 (SCH-23390) and D-2 (spiperone) antagonists with the low and high doses of METH. Eclopikid (0.5 mg/kg) alone decreased NT release >20% and completely masked the dose effect of METH. SCH-23390 (0.5 mg/kg) alone had no effect on basal NT release, but the combination of D-1 blockade with 15 mg/kg METH increased NT release to ~150% of control. These results demonstrate that low dose METH on NT release is mediated through stimulation of D-2 receptors, while the lack of effect of high dose METH is due to D-1 activation. (Supported by USPHS grants DA 34471 and DA 06809.)

286.6 INCREASED DOPAMINE D1 RECEPTOR SITES OF THE SUBSTANTIA NIGRA RETICULATA FOLLOWING INTERMITTENT ADMINISTRATION OF METHAMPHETAMINE. S. Zhang and J.A. Andreula. Department of Biological Sciences, Hunter College, NY.

We have assessed effect of intermittent administration of methamphetamine (METH) pretreatment on haloperidol-induced effects on neostriatal ventral and dorsal nucleus accumbens (VNA and DNa) dopamine (DA) and receptor levels. METH was administered to rats (N=10 each) for 7 consecutive days. Treatment with METH did not affect neostriatal "spiperone (D2)" binding sites, but METH pretreatment significantly increased haloperidol-induced increases of D1 receptor sites in ventralostriatal caudate-putamen (vCPu) and nucleus accumbens (N.Acc). In contrast, treatment decreased "spiperone (D1) (1nM)" binding with the N.Acc. Haloperidol treatment of METH-pretreated rats significantly decreases the vCPu, anterior Cpu (aCpu) and N.Acc. in the midbrain ventral substantia nigra reticulata (vSNr); METH pretreatment increased D1 receptor sites (20% above control). In addition, the haloperidol treatment of saline-pretreated rats increased D1 receptor sites of the VNA 58% relative to controls. METH pretreatment enhanced haloperidol-induced changes in striatal preproenkephalin (PPE) and proenkephalin mRNA abundance. The present results show that pretreatment with METH increases dopamine D1 receptor sites in the midbrain substantia nigra pars reticulata and potentiates haloperidol-induced alterations of dopamine receptors and neuropeptide mRNA abundances in the caudate-putamen and nucleus accumbens of the rat brain.


Three experiments were conducted to investigate the striatum dopamine (DA) release in response to M-stimulated decline rapidly over time after probe insertion. In all three experiments, M exposure occurred for one group three times at two-hour intervals, with the first probe occurring 2 hr after probe insertion. Independent of route of administration (1 mg/kg M at 20 μM of M for 10 min via the microdialysis probe), the DA peak elicited by the third probe was significantly less than the peak elicited by the first exposure, 4 hr earlier. This reduction in the response to M was a function of time post probe insertion, and not of probe M exposure. When probes were inserted at 0700 hrs but the first M infusion occurred at 1100 hrs, striatal DA levels were as low as the 1300 hr peak in rats given infusions at 0900, 1100 and 1300 hrs. Conversely, when the probe was inserted 1 hr after the third ip M injection, the DA released by the third injection was considerably increased 2 hr after probe injection. Finally, ip M-induced peaks 24 hr after probe insertion were less than 10% of those seen 2 hr after the first probe insertion, and these much smaller peaks did not decline over repeated injection. It is concluded that the magnitude of M-stimulated striatal DA decline follows the first 24 hours following probe insertion.
METHAMPHETAMINE EFFECTS ON BRAIN ENERGY METABOLISM: COMPARISON BETWEEN EXTRACELLULAR LACTATE IN STRIATUM AND PREFRONTAL CORTEX. S.E. Hefta*; T.S. Whittingham; A.J. Douglas and B.K. Yamamoto of Departments of Psychiatry, Neuroscience, and Neurological Surgery, Case Western Reserve Univ., Cleveland, OH 44106.

A high dose of METH coadministered with 7-OH-DPAT (0.01 mg/kg) reduced systolic blood pressure and heart rate, thereby decreasing the blood pressure and heart rate to levels lower than those obtained with either agent alone. The decreased blood pressure and heart rate were accompanied by a decrease in plasma lactate levels in the striatum (STR) and a decrease in plasma lactate levels in the plasma of the same animals. However, the decrease in plasma lactate levels in the plasma of the same animals was not significantly different from the plasma lactate levels in the plasma of the same animals, as determined by the discriminative stimulus effects of METH and saline injected rats. The decrease in plasma lactate levels was also accompanied by a decrease in the number of animals that responded to the injection of saline. This decrease in responding to saline injection was not significant when the animals were tested in a cocaine self-administration paradigm. The decrease in plasma lactate levels was not accompanied by a decrease in plasma lactate levels in the plasma of the same animals, as determined by the discriminative stimulus effects of METH and saline injected rats. Further studies are needed to determine the role of lactate in the regulation of energy metabolism in the brain.

826.11

THE D1 AGONIST 7-OH-DPAT GENERALIZES TO THE DISCRIMINATIVE STIMULUS EFFECTS OF AMPHETAMINE IN RATS. R.A. Bies*; M.C. Bradley, J.E. Kleinberg, & M.B. Bardo. Psychology Dept, Univ of Kentucky, Lexington, KY 40506-0044.

Rats were first trained to discriminate between 1 mg/kg amphetamine and saline (ip) using a two-lever discrimination procedure. Following acquisition of the discrimination, generalization to a range of amphetamine doses (0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg) was examined in 4-min extinction tests. The degree of amphetamine-appropriate responding varied directly with the dose of amphetamine. Response rate decreased systematically with greater amphetamine doses. We then assessed the ability of the putative D1 agonist 7-OH-DPAT (0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg) to produce locomotion and sniffing and to reduce amphetamine-appropriate responding. In all groups, rats were tested to each dose; on one test the 7-OH-DPAT was injected ip and in the other test it was injected sc. Regardless of injection route, the highest doses of 7-OH-DPAT fully generalized to amphetamine. However, when 7-OH-DPAT injected sc occurred greater amphetamine-appropriate responding at lower doses than when injected ip. 7-OH-DPAT also produced a dose-dependent decrease in barpress rates with both injection routes. The D1 agonist eticlopride (0.01 and 0.05 mg/kg; ip) partially blocked the amphetamine-appropriate responding induced by 7-OH-DPAT. This latter result argues that the generalization of 7-OH-DPAT may be mediated, at least in part, by dopamine D1 receptors.

826.13

EFFECTS OF 7-OH-DPAT ON AMPHETAMINE-INDUCED STIMULANT BEHAVIORS AND CONDITIONED PLACE PREFERENCE. T.V. Khroyan; B.A. Baker; A.P. Fuchs and J.L. Nieswander. Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

Putative D1 agonists 7-OH-DPAT (0.01-0.1 mg/kg) produce a decrease in locomotion and sniffing and do not produce conditioned place preference (CPP). This study examined the effect of these doses on amphetamine-induced behaviors and CPP. Three 2-day conditioning trials were conducted over consecutive days. On one day of each trial, animals received an injection of either amphetamine (1.0 mg/kg) coadministered with 7-OH-DPAT (0.01-0.1 mg/kg) or saline and were placed into a compartment for 40 min. On the other day, animals were injected with saline and placed into a different compartment for 40 min. Locomotion, sniffing, and headbobbing were measured following the first and third drug injections. Following conditioning, CPP was assessed by recording the amount of time animals spent in each compartment after free-access to both. Amphetamine increased locomotion after the first injection, and this effect was attenuated by 0.03 mg/kg 7-OH-DPAT. Amphetamine-induced locomotion was decreased to a similar extent with doses of 7-OH-DPAT (0.01-0.1 mg/kg) and 7-OH-DPAT (0.01 mg/kg) coadministered with amphetamine. However, 7-OH-DPAT (0.01 mg/kg) coadministered with amphetamine did not produce CPP. These results suggest that 7-OH-DPAT potentiates the stimulant effects of amphetamine but alters the rewarding properties (supported by DA07730 and HHMI).

826.10

BEHAVIORAL SENSITIZATION TO 7-OH-DPAT: EFFECTS OF SELECTIVE DOPAMINE ANTAGONISTS. R.M. Metcalf; S. Fields; M. Langpole; M. Cecile; & J. Giovannini. Department of Psychology, Morehead State Univ., Morehead, KY 40351.

Repeated treatments with the dopamine D1, D2-type agonists, bromocriptine and quinpirole, respectively, have been shown to produce development of behavioral sensitization. This sensitization effect, however, may be blocked by the co-administration of either DA or D2 antagonists. The primary purpose of this study was to determine if DA D1- and D2-type antagonists would also block the development of sensitization to the putative DA D1 agonist, 7-OH-DPAT. In Exp. 1, rats were exposed to 2 hr in photocell activity arenas daily after treatment with 7-OH-DPAT (0.01, 0.1, or 1.0 mg/kg). All doses initially inhibited activity, but with repeated treatment, the 1.0 mg/kg dose resulted in sensitization. No cross-sensitization to cocaine was observed. In Exp. 2, rats were co-administered 7-OH-DPAT (1.0 mg/kg) and either the D1-type antagonist, eticlopride (ETIC, 0.3 mg/kg) or the D2-type antagonist, SCH 23390 (SCH, 0.2 mg/kg), and tested for activity. Both antagonists significantly suppressed activity and prevented the progressive 7-OH-DPAT-induced increase in activity over sessions. ETIC, but not SCH, also blocked the development of sensitization. This is, after a 7-OH-DPAT challenge injection, rats pretreated with ETIC and 7-OH-DPAT did not differ from vehicle pretreated rats. Rats pretreated with SCH and 7-OH-DPAT, however, were significantly more active than rats pretreated with only 7-OH-DPAT.

826.12

DOSE-DEPENDENT EFFECTS OF THE D3-PREFERING AGONIST 7-OH-DPAT. J.L. Neiswanger; T.V. Khroyan, and D.A. Baker. Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

Dose-dependent effects of 7-OH-DPAT (DPAT) on motor behaviors and conditioned place preference (CPP) were assessed. Two-day conditioning trials were conducted. On one day, animals received one of 8 doses of DPAT (0.5 mg/kg, sc) and were placed into a distinct compartment for 40 min. On the second day, they were injected with saline and immediately placed into a different compartment for 40 min. Three trials were conducted over consecutive days. Locomotion, sniffing, and yawning were measured following the first and last injection of DPAT. CPP was assessed by recording the amount of time animals spent in each compartment after free-access to both for 15 min. DPAT produced a U-shaped dose-dependence in locomotion and sniffing with the greatest increases observed at 0.01 and 0.03 mg/kg. DPAT also produced an inverted U-shaped dose-dependence in locomotion and sniffing with the greatest decreases observed at 0.01 and 0.03 mg/kg. DPAT-induced yawning was sensitized at a dose of 0.1 mg/kg. The 5 mg/kg dose of DPAT produced CPP. None of the other doses (0.003-1 mg/kg) produced CPP, and in fact there was a trend for place aversion at 0.3 mg/kg. DPAT has a 100-fold greater affinity for D3 receptors relative to D2 receptors. Thus, it is possible that the low doses (0.01-0.1 mg/kg) that increased yawning and decreased locomotion and sniffing may preferentially occupy D3 receptors. Furthermore, these putative D3-prefering doses of DPAT do not produce CPP and may produce place aversion. (Supported by DA07730)

826.14

REPEATED PHENYCICLINE TREATMENTS ACTIVATE CHOLECSTYKININ RECEPTORS IN RAT BRAIN. H. Shibuya*; K. Yamada, T. Yoshikawa, T. Nabessa, M. Torai; 1:Department of Neuropsychiatry, Tokyo Medical and Dental Univ., 1-5-45, Yoshima, Bunkyo-ku, Tokyo, 113, Japan; 2: Nogoya Univ., School of Medicine, 45, Tsurumachio, Showa-ku, Nogayo, 466, Japan.

Reported findings on cholecystokinin(CCK) immunoreactivity in the postmortem brain and cerebrospinal fluidings. CCK is a ubiquitous neurotransmitter in the mammalian brain. Meanwhile, the role of phenycyclidine (PCP) is known to produce positive and negative psychotic symptoms in human. We assumed the PCP treated rat as the schizophrenia animal model and determined the CCKmRNA and CCK immunoreactivities in various brain areas in order to investigate the functional state of CCK in schizophrenia. Single administration of PCP (7.5mg/kg, ip) promptly and transiently decreased CCK immunoreactivities in a manner dependent on the hippocampus and parieto-occipital cortex. However, repeated administrations of PCP increased the synthesis of CCK. These findings would suggest that CCK systems are hyperactive in schizophrenia and play roles to produce some schizophrenic symptoms.
ACUTE AND LONG-TERM NEUROCHEMICAL EFFECTS OF METHCATHINONE, A NEW STIMULANT OF ABUSE. M.P. Ogilvie, J.W. Gibb, and D.R. Hanson* (Dept. of Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

Methcathinone (CAT), a synthetic derivative of cathinone, emerged as an illicit drug of abuse in the U.S. during the early 1990s. Because of ease of synthesis, CAT is preferred by some over cocaine or methamphetamine (AMPH). Multiple doses of CAT were shown to be toxic to brain dopamine (DA) and 5-hydroxytryptamine (5HT) neurons (Manuello et al., Soc Neurosci 419, 1994). In striatum, a single dose of CAT decreases the activity of cytophilic hydroxylase (TH), the rate-limiting enzyme in the synthesis of 5HT. To characterize further the time course of these effects, rats were given 4 doses of CAT (30 mg/kg) 4 h apart, and sacrificed 72 h or 30 following the last dose. In the 72 h group, striatal TH activity was decreased. The activity of striatal tyramine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, was also decreased. Stratal concentrations of DA and 5HT and their metabolites were reduced as well. Both TH and TPH activities returned to control by 30 h after treatment. Because drug-induced increases in mesostriatal dopaminergic activity elevate striatal levels of neurotensin, the response of this peptide to CAT was assessed and compared to METH, a known releaser of DA. Rats were given either 4 doses of CATH (30 mg/kg), METH (15 mg/kg), or saline 4 h apart, and sacrificed 18 h after the last dose. Both METH and CAT increased striatal levels of neurotensin to greater than 200% of control. This data demonstrates that the responses neurotensin parameters in the striatum. These effects do not appear to be permanent. Since striatal neurotensin levels were elevated by CAT, it is possible that the neurochemical response is due to CAT-induced DA release. (supplied by DA 00422 & DA 00869)

S(-)METHCATHINONE AS A DISCRIMINATIVE STIMULUS: EFFECTS OF OTHER CNS STIMULANTS.

Methcathinone ("Cat") is a CNS stimulant that is very significant as a drug of abuse in the former Soviet Union. It also has appeared on the clandestine market in the US and has recently been classified as a Schedule I substance. We have shown previously that racemic methcathinone and its two optical isomers all produce both amphetamine- and cocaine-like discriminative stimulus effects in rats trained to discriminate S(+)-amphetamine and cocaine, respectively, from saline. In those studies, S(-)methcathinone was almost twice as potent as S(-)amphetamine and more than eight times more potent than cocaine. In the present study, using rats, S(-)methcathinone (0.5 mg/kg, i.p., 15 min pretreatment) was employed as the training drug in a two-lever discrimination task. Once established, the S(-)methcathinone stimulus was shown to have a rapid onset to action (5 min pre-session injection interval=89% drug-lever responding) and a duration of effect of approximately 90-120 min. In tests of stimulus generalization, the S(-)methcathinone-stimulus (ED30=0.11 mg/kg) generalized to R(-)methcathinone (ED50=0.29 mg/kg), S(+)-amphetamine (ED50=0.20 mg/kg), and cocaine (ED50=1.47 mg/kg). Thus, the present results re-confirm our previous conclusion that S(-)methcathinone is a very potent CNS stimulant with amphetamine- and cocaine-like effects.


PHARMACODYNAMIC ANALYSIS OF KETAMINE ACTION IN SCHIZOPHRENIA: D. Medoff*, A.C. Lahn, H.H. Holcomb, M. Zhao, C.E. Pribe, C.A. Tammenga. MPRC, University of Maryland School of Medicine, and the Department of Mathematical Sciences, Johns Hopkins University.

The noncompetitive NMDA antagonist ketamine produces a short lived discrete activation of psychotic symptoms in schizophrenia. To study regional neural activity that corresponds to ketamine administration, we administered 3mg/kg of ketamine to five medicated schizophrenic inpatients and measured regional cerebral blood flow (rCBF) using H215O and positron emission tomography (PET) before (x3) and at seven time points after ketamine administration. The post ketamine administration time points ranged from 6 to 66 minutes. After image registration, a 12 mm three dimensional Gaussian filter was used to smooth the data. Data were analyzed using the Statistical Parametric Mapping (SPM96) software program, MPRC Brigham Unit, Hammmershmit Hospital. Significant patterns of blood flow change over time were detected in several regions. The lingual gyrus shows an immediate drop in blood flow at six minutes and a switl retard. To baseline by sixteen minutes. The hippocampus also shows an immediate drop in blood flow, but a more gradual return to baseline at 56 minutes. The anterior cingulate, inferior frontal cortex, thalamus, and the cerebellum all showed increases in blood flow, but in distinctly different patterns. It is noteworthy that the areas of ketamine-affected rCBF are circumscribed, limited in number, and much more restricted than the distribution of NMDA/PP receptors. Based on the localization of blood flow response ketamine in these subjects, we suggest that limbic brain regions are important in mediating the behavioral actions of ketamine. These data implicates an abnormality of glutamatergic transmission in psychosis.

AMYGDALA LESIONS THAT BLOCK SENSITIZATION TO BROMOCRIPTINE FAIL TO BLOCK SENSITIZATION TO MCCAIN. E.A. Guarraci, and R.A. Wise Center For Studies in Behavioral Neurobiology, Concordia Univ., Montréal, QC, CANADA H3G 1M8.

Repeated intermittent administration of the dopamine (D2) agonist bromocriptine (BRO; 5.0 mg/kg, IP) causes progressive increases in sensitivity to the locomotor-stimulating actions of the drug in rats; this "sensitization" is context-specific, such that rats repeatedly given BRO in one environment show no evidence of sensitization when tested in a different environment. We now report that electrolytic lesions of the basolateral amygdala (BA) can eliminate BRO's sensitization without altering sensitivity to the acute stimulant effects of the drug, suggesting that the drug-environment associations that contribute to the sensitization process are at a more distal level in the amygdala. Similar lesions of the amygdala fail to block the progressive increases in locomotion that occur when MK-801 (0.25 mg/kg, IP) is co-administered with BRO, under conditions that do not produce sensitization to MK-801 alone. However, there is no evidence of sensitization when animals (lesioned or sham-lesioned) accustomed to receiving the combination of MK-801 plus BRO subsequently receive either drug alone. Thus it is the absence of MK-801 in animals accustomed to the combination of drugs that precludes expression of sensitization. These findings suggest that MK-801 makes responses learned under its influence "discriminative cue-specific", and that such cues can facilitate experience-dependent changes in drug sensitivity even when the amygdala is damaged.

FASCICULUS RETROFLEXUS LESIONS INCREASE SELF-ADMINISTRATION OF AMPHETAMINE. Carol A. Murphy and Marion Murphy Centers for Neurology, University of Pennsylvania, and Hahnemann University, 3200 Henry Avenue, Philadelphia, 1PA 19129.

Disruption of the efferent pathway of the habenular nucleus, the fasciculus retroflexus (FR), produces behavioral and physiological changes that reflect a state of increased arousal, including increased open field activity and chronically elevated basal levels of corticosterone. Within a normal population of Sprague-Dawley rats, similar responses to stress are positively correlated with increased self-administration of amphetamine. Because habenular projections through the FR tonically inhibit the activity of dopaminergic (DA) neurons in the ventral tegmentum, and intake of psychostimulant drugs is tightly linked to forebrain DA activity, we tested whether rats with FR lesions would self-administer higher doses of amphetamine. Two months after receiving either FR or sham lesions, female Sprague-Dawley rats were prepared with catheters in the external jugular vein and tested for intravenous self-administration of d-amphetamine sulfate (10ug/injection) in both nose-poke and lever-press response paradigms. Rates of amphetamine self injection and the incidence of behavioral stereotypy were assessed during daily 1-hour sessions. Our results show that FR-lesioned animals initially self-administered greater amounts of amphetamine than sham controls over the first several days of testing but that their drug intake gradually decreased to near-control levels. Stereotypic responses to amphetamine were also greater after FR lesion and remained elevated with respect to controls for the duration of the experiment. These results suggest that habenular regulation of DA reward systems may normally provide an important negative feedback mechanism which moderates the intake of psychostimulant drugs. Supported by NIMH award MH53635-01.
827.1


Previous electrophysiological experiments demonstrated that 5-hydroxytrypamine (5-HT1A) agonists inhibited spontaneous pyramidal cell activity in anesthetized rats. Since all the 5-HT1A agonists that have been tested act as agonists post-synaptically, it is important to know whether these 5-HT1A agonists inhibit spontaneous activity under conditions in which 5-HT neurons are activated. So we examined the effects of 5-HT1A agonists on single-unit activity of hippocampal CA1 pyramidal neurons in unanesthetized, unrestrained rat. Subchronic administration of the selective 5-HT1A agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), buspirone, ipsapirone, and a2-adrenergic agonists (dihydroxyphenylisobutylamine (DHPIA)) inhibited the firing of 5-HT neurons.

The present study provides the following results. First, the effect of 5-HT1A agonists on single-unit activity of hippocampal CA1 pyramidal neurons in unanesthetized, unrestrained rats. Second, the effect of 8-OH-DPAT (10 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats. Third, the effect of buspirone (10 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats. Fourth, the effect of ipsapirone (30 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats. Fifth, the effect of DHPIA (30 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats.

The results of the present study suggest that 5-HT1A agonists inhibit the firing of 5-HT neurons in unanesthetized, unrestrained rats. The effect of 5-HT1A agonists on the firing of 5-HT neurons in unanesthetized, unrestrained rats was stronger than that observed in anesthetized rats. The effect of 8-OH-DPAT (10 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats was stronger than that observed in anesthetized rats. The effect of buspirone (10 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats was stronger than that observed in anesthetized rats. The effect of ipsapirone (30 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats was stronger than that observed in anesthetized rats. The effect of DHPIA (30 mg/kg i.p.) on the firing of 5-HT neurons in unanesthetized, unrestrained rats was stronger than that observed in anesthetized rats.

827.2


MKC-242 (8-S-(2S)-(1S)-1-benzodioxo-2-(3-hetarylamino)propyl-1,3-benzodioxol (HCI)) is a potent and selective 5-HT1A receptor agonist. It showed anxiolytic-like and antidepressant-like effect at very low doses (0.0025-3 mg/kg, p.o.) in several animal models. MKC-242 is structurally different from aziphrone. In the present study, we investigated two different profiles of MKC-242 from aziphrone.

First, the effect of MKC-242 on the immobility time in the forced swimming test in male mice was investigated comparing with buspirone and tansdepiperazine (3.0 mg/kg, p.o.). MKC-242 was significantly different from aziphrone.

Second, the effect of MKC-242 on the shock-induced fighting behavior in male mice was investigated comparing with buspirone and tansdepiperazine (3.0 mg/kg, p.o.). MKC-242 was significantly different from aziphrone.

In conclusion, MKC-242 showed more potent antidepressant-like effect and longer-lasting anxiolytic-like effect comparing with buspirone and tansdepiperazine. These characteristics might be due to the structural difference and pharmacodynamic difference from aziphrone.

NMDA receptor antagonists are as efficacious as clinically active antidepressants (AD) in both preclinical behavioral paradigms sensitive to ADs and in animal models of depression. Moreover, 10-14 d continuous administration of an AD is required to produce a decrease in the expression of the NMDA receptor complex in rodents. Specifically, chronic AD administration results in a 2-4 fold reduction in the potency of glycine to displace [3H]-N-methyl-D-aspartate (NMDG)-3H] in the glycine recognition site of the NMDA receptor complex. In rodents, chronic AD treatment results in a 40-100% reduction in the proportion of high affinity, glycine-dependent NMDA receptors to the lowest affinity, glycine-independent NMDA receptor complex. These effects are not observed following chronic administration of structurally related non-AD treatments. A limitation of these earlier studies is that adaptation of the NMDA receptor complex was observed following AD administration in naïve animals. We therefore tested the hypothesis that animal models of depression would result in AD-reversible adaptation of the NMDA receptor complex. We administered imipramine (IM - 10 mg/kg x 28 days) to rats subjected to CMS. As previously reported, 28 days CMS resulted in reduced consumption of a sucrose solution. This was reversed by administration of IM. Moreover, we now report CMS results in adaptation of the NMDA receptor complex and that this adaptation is reversible by chronic IM treatment. These data lend support to the hypothesis that AD activity is associated with adaptation of the NMDA receptor complex and that "depression" or "anhedonia" in animals is associated with an AD-reversible dysfunction of the NMDA receptor complex.

LYSOSOMOTROPIC DRUGS SLOWLY ACUMULATE IN HUMAN BRAIN TISSUE. RELATIONSHIP TO THERAPEUTIC LATENCY OF ANTIDEPRESSANT AND NEUROLEPTIC DRUGS. K. Henningfeld, W. Ratafia P. Riederer. Dept. of Psychiatry, University of Würzburg, 67000 Würzburg, Germany.

The mechanism of therapeutic latency of antidepressant (AD) and neuroleptic (NL) drugs is not clearly understood. Current hypotheses include slow adaptation of receptors or transport processes. Here, we present a hypothesis explaining therapeutic latency by slow accumulation of the drugs in acidic intracellular compartments. We have studied the pharmacodynamics of amantadine, a lysosomal model substance. Its fast therapeutic response is mediated by fast access to cell surface receptors. However, it slowly accumulates in human brain tissue. Half-maximal and plateau concentrations are reached after 6 and at least 70 days of treatment, respectively. The concentration in brain tissue relative to CSF is about 150. The high storage capacity of brain tissue is probably related to lysosomal properties of amantadine. This means that amantadine, as other lysosomal substrates, is trapped by proton-driven organelle membranes in the cells and may not diffuse to extracellular processes that require an acidic milieu, such as the proton-driven transport of monoamines into synaptic vesicles. The mean daily oral dose of amantadine is low compared to the high storage capacity of brain tissue. Studies show that the storage of amantadine in brain tissue can explain the observed therapeutic latencies in many psychiatric diseases. In addition to the role of amantadine, the hypothesis can also explain the slow accumulation of other drugs and drug combinations, such as antipsychotics. This means that antidepressants and neuroleptics are slowly accumulated in the brain and thus the therapeutic latency is explained by the slow accumulation of the drugs.

QUINAPRINE PARTIALLY REVISED THE RHYTHM SLOWING AND FORCE DECREASING EFFECTS OF CLOzapine ON Rats' YEOMOVEMENT DURING LAPPING BEHAVIOR. S. Das* and S.C. Fowler. Dept. of Pharmacology & Toxicology, Univ. of Kansas, Lawrence, KS 66045.

In order to investigate the putative role of serotonin receptor blockade in the slowing of rats' licking behavior induced by clozapine (Dase & Fowler, Psychopharmacology, 1995), the nonselective serotonin agonist quinaprine was administered to rats treated acutely with clozapine. Thrity rats (n=29) were trained to lap water from a force-sensing dish in a Skinner box. Orthogonally, the rats were treated with clozapine (1.5-10 mg/kg, ip, 20 min) and with quinaprine (0.5, 1.0, 2.0 mg/kg, ip, 30 min) significantly quickened the licking rate at the lowest doses. Clozapine (1.5, 3.0 mg/kg, ip, 20 min) robustly reduced licking rate, and quinaprine coadministration partially, but significantly reversed the clozapine-induced behavioral slowing. The results were consistent with the hypothesis that serotonin receptors are involved in clozapine's effects on rats' paltry behavior. Supported by MH64329.

SAFETY AND EFFICACY OF THE CCK4 ANTAGONIST CI-988 IN GENERALIZED ANXIETY DISORDER. John F. Spainak, Jerome R. Costa, Judith Bannister, Scott Haro, Allison MacPherson, and Neal R. Cutler. California Clinical Trials, Beverly Hills, CA 90211 and Parke-Davis Division of Warner-Lambert, Ann Arbor, MI

CI-988 is a novel peptidyl CCK antagonist with affinity for brain CCK1 and gastrin receptors. This study investigated the efficacy of this compound in patients with generalized anxiety disorder (GAD). We report results from one site (n=52) in a multicenter study (n=98). Patients were randomized to a double-blind, placebo-controlled, parallel group study. Pathological scores of anxiety were randomized to 300 mg/day CI-988 and 16 to placebo. Twenty-nine patients completed all four weeks of treatment. The primary efficacy measures were: the Hamilton Rating Scale for Anxiety (HAM-A) and the Clinical Global Impression (CGI) scale. Patients on CI-988 showed a greater decrease (p=0.06) in HAM-A scores than did patients on placebo at the end of the study, with mean change of -6.19 in patients on CI-988 and -0.64 in placebo. The HAM-A change favoring CI-988 was primarily due to improvement in somatic symptoms of anxiety. There were no significant differences on the CGI between the patients treated with CI-988 versus placebo (mean changes of -0.88 and -0.34, respectively, in impression of severity score, and mean changes of 0.92 and 0.94, respectively, in impression of change scores). All adverse events were rated mild or moderate. One patient on CI-988 discontinued due to moderate abdominal pain; two patients on placebo discontinued for personal reasons. Our findings indicate that CI-988 given for four weeks has a potential anxiolytic effect, though the small sample size limits its certainty. These findings were not reflected in the overall multicenter results: one center favored placebo and the other favored neither group. Given the acceptable tolerability of CI-988 seen here, testing of higher oral doses in patients with GAD may be warranted.


An add-on anxiolytic effect is desirable for neurolcpsychic drugs, as emotional disturbances are frequently accompanying symptoms in schizophrenia patients. The non-classical neuroleptic sertindole shows potent anxiolytic-like effects in rodents and the mannequin®. In this in vitro study we have compared the profiles of haloperidol and clozapine with the newer anxiophysics sertindole, risperidone, olanzapine, seroquel and ziprasidone in 3 animal models, i.e. facilitation of exploratory behaviour of rats in a two-compartment black and white box (BW), inhibition of forced-wheel-induced ultrasonic vocalization in adult rats (USV) and inhibition of isolation-induced aggressive behaviour in male mice (AGGR). Sertindole is the only compound with an anxiolytic-like profile in the BW test (i.e. increased exploration of the white compartment relative to the black). Olanzapine shows an anxiolitic-like profile, and the other compounds are inactive or weakly anxiolitic-like. Cisenapline, risperidone and olanzapine inhibit USV, whereas sertindole and ziprasidone are inactive. Haloperidol is also inactive, even at dosages that inhibit locomotor activity markedly. Sertindole, clozapine, riseridone and seroquel inhibit AGGR, whereas olanzapine is inactive. Haloperidol is also inactive, even at dosages that inhibit locomotor activity of non-aggressive mice markedly. In conclusion, the classical neuroleptic haloperidol is inactive, and the newer anxiophysics and clozapine constitute a heterogeneous group with regards to anxiolytic-like effects in rodents.


A SINGLE INJECTION OF IBOGANE PRODUCES SIGNIFICANT EFFECTS IN CARTOCISTEROlONE LEVELS AND SELECTIVE CHANGES IN THE DOPAMINIC SYSTEM IN RAT BRAIN. S. E. G. D. Newton, W. Slikker, Jr., B.B. Rothman and M. H. Baumann. Neurochemistry Laboratory, Division of Neurotoxicology, NCTR/DFDA, Jefferson, AR 72079, and Addiction Research Center, NIDA, Baltimore, MD 21224.

Recently, we reported that a single injection of ibogaine (IBG) produced significant alterations in nictin oxide synthase activity and monoamines levels in mouse brain. Other reports indicated that IBG has no effect on the opioid receptors (kappa and sigma). The neurochemical mechanism(s) and its role as a treatment medication for drug addiction is still unclear. The present study was designed to evaluate the effect of IBG on various brain regions and the dopaminergic system. Adult SD rats were dosed with 50 mg/kg IBG, ip, and sacrificed 15, 30 minutes, 1, 2 and 24 hr later. Trunk blood was collected for hormone levels. Brain tissues were dissected and analyzed. IBG produced significant elevations in corticosterone liver levels and decreases in dopamine (DA) concentrations in striatum decreased significantly at 30 minutes, 1 and 2 hours after drug administration, however, it returned to control levels at 24 hr. DA metabolites and DOPAC and HVA concentration increased in a time dependent manner up to 2 hr. At 24 hr DOPAC concentrations were below control values whereas HVA returned to control levels. Concentrations of serotonin and its metabolite 5-HIAA were decreased only for serotonin. These findings suggest that a single injection of IBG can produce significant elevations of corticosterone and depletions of DA in a time-dependent manner. Future experiments will determine if these effects are mediated through the kappa or sigma receptors.
87T.13
THE PUTATIVE 'ENDABE' IOIGANE INTERACTS WITH THE PEP AND SIGMA BINDING SITES IN RAT BRAIN. Yoshifumi Ishibash* and Syoichi Ueda, University of Miami School of Medicine, Miami FL 33101 and Division of Neurotoxicology, NCTR/FDA, Jefferson, AR 72079.

Although the alkaloid ibogaine is a potent hallucinogenic agent some indications suggest that it may be useful for the treatment of opioid and cocaine addiction. The underlying mechanism(s) mediating the ibogaine effects remain unclear. In the present study we investigated the interaction of ibogaine with the PEP site located in the ionophore of the NMDA receptor complex, with the NMDA receptor binding site, and with sigma binding sites. In well-washed membrane preparations of rat cortex and cerebellum, the PEP sites were labeled with [3H]MK-801 or [3H]TCP, and the NMDA receptor with [3H]CGP 39653. The sigma-1 and sigma-2 binding sites were labeled with [3H]DTG, and [3H]DTG, respectively. Results indicated that ibogaine interacts with High and Low affinity PEP binding sites in the cortex: Kd = 0.01-0.05 μM, Ki(2) = 2.4 μM. In contrast, ibogaine (>100 μM) had no affinity for [3H]TCP binding sites (cortex and cerebellum). The affinity of ibogaine for sigma-1 and -2 binding sites in cortex and cerebellum ranged from 1.5 - 3 μM. Since NMDA receptor antagonists (e.g., MK-801) are thought to attenuate opioid withdrawal symptoms and cocaine sensitization, it is possible that binding of ibogaine to the PEP sites contributes to its potential 'endabes' properties. In turn, ibogaine interaction with sigma binding sites may be associated with its adverse effects.

87T.15
EFFECTS OF CHRONIC LITHIUM, VALPROIC ACID, AND CARBAMAZEPINE ON THE EXPRESSION OF THE PKC SUBSTRATE MARCKS IN IMMORALIZED HIPPOCAMPAL CELLS. D.G. Watson*, R.K. McNamara, and R.H. Leson*, University of Florida College of Medicine, Gainesville, FL 32610.

Studies in our lab and others support a proven role of PKC in mediating the effects of chronic lithium in brain (Manji and Lenox, 1994). We have previously reported that chronic lithium alters the expression of a major PKC substrate, MSK-1 (a Myristoylated Alkaline Membrane (Kinase Substrate)), in both rat hippocampus (Leson et al., 1992), and in immortalized hippocampal cells in culture (Lenox et al., 1993). In addition, exposure to phenol esters also induces a rapid down-regulation of MARCKS protein (PKC dependent mechanism) in cerebellar and hippocampal cells (Watson et al., 1994). In the present study we have compared the effects of chronic exposure to the anti-manic agents lithium, valproic acid, and carbamazepine, on the expression of MARCKS protein in immortalized hippocampal HN33 cells. HN33 cells were grown in non-tissue-damaged DMEM media supplemented with 20% fetal bovine serum. Following exposure to lithium chloride (10-100mM), sodium valproate (0.5-1.5mM), and carbamazepine (2.5-100mM), cells were collected, fractionated into soluble and membrane fractions, and MARCKS protein assayed by western blot analysis. Both lithium chloride and sodium valproate exposure produced a significant dose-dependent reduction in MARCKS protein in both the soluble and membrane fractions, following long-term exposure (3-7 days). No alterations in MARCKS protein levels were observed following acute exposure to either agent. In contrast, no changes in MARCKS protein levels were detected following long-term exposure to carbamazepine. Regulation of MARCKS protein may represent a pharmacological property shared by mood stabilizers with therapeutic efficacy in the prophylactic treatment of manic-depressive illness. (Supported by NIMH grant MH43429).

87T.16
DANTROLENE DIMINISHED FORELIMB FORCE EMISSION IN A PRESS-WHILE-LICKING BEHAVIORAL TASK. J.A. Stanford* and E.C. Fowler, Dept. of Human Development, Univ. of Kansas, Lawrence, KS 66045.

The peripherally acting striate muscle relaxant, sodium dantrolene, was evaluated as a potential means for modeling reduced muscle tone in freely behaving rats. Rats were trained to use a single forelimb to exert continuous downward pressure on a force-sensing operandum, and water reward made available to the rat as long as forelimb force was maintained above 20g. Dantrolene (5.0, 7.5, 10.0 mg/kg, ip., 45 min before session) significantly and dose-dependently reduced the rats force output during the hold, but not the initiation segment of forelimb responses. Fourier analysis of the hold segment force-time records indicated that dantrolene diminished power above 5 Hz, but did not slow the oscillatory phenomena quantified by this method. Time on task was not affected by these doses of dantrolene. Together the data suggest that dantrolene can reduce striate muscle force production at doses that have negligible motivational consequences. Supported by MH43429.

87T.17
PROGESTERONE METABOLISM IS REGULATED BY CARBAMAZEPINE VIA EFFECTS ON 5A-REDUCTASE IN C6 GLIOMA AND NEURONAL/STAMINA CELLS. B.S. Fann, H.K. Maril and W. Lechaker, Section of Clinical Pharmacology, ETB, NIH, NIH, Bethesda, MD 20892.

Despite the widespread use of carbamazepine (CBZ) in the treatment of both neurologic and psychiatric disorders, its mechanisms of action remain to be elucidated. In recent years, considerable research has demonstrated significant effects of neuroactive steroids on the regulation of neuronal excitability. It is thus noteworthy that we have previously demonstrated that CBZ increases the steady-state levels of 5-Dehydroepiandrosterone (DHEA) in a time- and dose-related manner in C6 cells. Although preliminary evidence suggests that these effects may be mediated via peripheral, the role of CBZ in non-neural tissue has also been postulated to interact with 5a-reductase, the enzyme which converts progesterone to 5a-dihydroprogesterone (DHP). Since DHP plays an important role in regulating androgenic activities, we investigated the effect of CBZ on progesterone metabolism in both cultured rat C6 glioma cells and in neuroblastoma cells (since 5a-reductase activity is higher in neurons). We have found that CBZ alters 5a-reductase activity and therefore the 5a-progesterone conversion rate in both C6 cells and neuroblastoma cells. The mechanism(s) of 5a-reductase inhibition and the potential differences of progesterone conversion in neuronal and glial cells is currently under investigation.

87T.18
NON-COMPETITIVE NMDA ANTAGONISTS IMPAIR SPATIAL DELAYED ALTERNATION PERFORMANCE IN RATS: REVERSAL BY ANTIPSYCHOTICS. Anita Verma* and Rita Moghadam, Department of Psychiatry, Yale University School of Medicine, VA Medical Center 116A/2, West Haven, CT 06516.

In the present study, the effect of non-competitive NMDA antagonists on a prefrontal cortex (PFC) sensitive task was examined using a spatial delayed alternation (SDA) paradigm in rats. In addition, in vivo microdialysis was used to assess the effect of exposure to ketamine on extracellular dopamine levels in PFC and striatum. The non-competitive NMDA antagonists, ketamine (10, 20 and 30 mg/kg) and MK 801 (0.1 and 0.5 mg/kg), impaired the SDA performance as illustrated by the decrease in the number of correct responses during sessions with 15 sec inter-trial interval, as compared with the corresponding vehicle-treated control group. A significant increase in extracellular levels of dopamine was observed in PFC as compared with striatum following ketamine (30 mg/kg) administration. The disruptive effect of ketamine on SDA performance was reversed by haloperidol (0.1 mg/kg) but not by SCH 23390 (0.1 mg/kg). Administration of ketamine also induced partial reversal of ketamine-induced impairment of spatial working memory performance. These data suggest impairment of PFC-sensitive SDA performance by non-competitive NMDA antagonists. A modulatory role for dopamine receptor subtypes in ketamine-induced disruption of spatial working memory is also suggested.
828.1 NEUROPHYSIOLOGICAL AND BEHAVIORAL EVALUATION OF TREMBLER MICE BETWEEN 1 AND 2 MONTHS OF AGE. J.A. Grazer* and A.K. Vag, Dept. of Pharmacology, Cephalon, Inc., West Chester, PA 19380.

The Trembler mouse (heterozygous TR-J, Jackson Laboratories) is considered a model of Charcot-Marie-Tooth (CMT) disease. Both humans with CMT and Trembler mice have hereditary CMT2 gene (Suter et al., 1992, Nature 359:387-390). Functional impairment of TR-J and wild type littermates was assessed at 37, 51, and 65 days of age to determine whether the relative age of onset and progression of the pathology in man and mouse is similar. Behavioral performance was evaluated by detecting the latency for mice to traverse a 60 cm x 10 mm rod. Electrophysiological measurements included conduction velocities (CV) and amplitudes of sciotic, tibial, and sural nerves. Behavioral and electrophysiological deficits in Trembler mice were apparent at 37 d of age and did not markedly progress (see table; p<0.05 vs control). Behavioral impairment in TR-J mice was induced by an increased latency to traverse the rod. Conduction velocity deficits for sural and tibial nerves were similar to those shown below for the sciatric n. Amplitude measures for all three nerves were reduced by 60% or more in TR-J mice and did not significantly change over time.

<table>
<thead>
<tr>
<th>Age</th>
<th>Rod Latency (s)</th>
<th>Sciatric CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37d</td>
<td>5.5±0.9*</td>
<td>5.2±0.3*</td>
</tr>
<tr>
<td>51d</td>
<td>5.0±1.0</td>
<td>4.6±0.3*</td>
</tr>
<tr>
<td>65d</td>
<td>6.9±0.3*</td>
<td>6.0±0.4*</td>
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In contrast to these data, human neurological symptoms of CMT typically are not seen until late childhood and progress to moderate disability by age 30. We conclude that the Trembler model is inappropriate for assessing the ability of agents to reverse the neurologic deficits of CMT. However, since neurologic deficits in the Trembler mouse appear early and fail to progress, this model is inappropriate for evaluating agents intended to affect the pathology of CMT.


Cultures of mouse ventral spinal cord express choline acetyltransferase (ChAT), detected as enzyme activity in homogenates or immunocytochemically in individual neurons. Glia-neuron interactions are important for neuronal survival and cholinergic expression have been demonstrated in this system. We studied cholinergic expression of cultured neurons grown on cerebral cortical glia derived from either normal or TS16 trisomic animals. The TS16 trisomy is of particular interest in that it is homologous to the human Trisomy 21 (Down syndrome). The ChAT enzymatic activity of neurons grown on TS16 glia was reduced to 25% of control values. Total neuronal counts were no different in the two conditions. TS16 neurons grown on TS16 glia expressed ChAT levels 37% of normal. When TS16 neurons were grown on normal glia, ChAT levels were 63% of normal. This was significantly greater than the values for TS16 neurons growing on TS16 glia. We hypothesize that glial cells derived from TS16 animals are either deficient in some cholinergic trophic agent or, more probably, produce some material with a negative effect on neuronal function with some specificity for cholinergic neurones.


It has been postulated that chemokines mediate cell migration to the central nervous system (CNS) during pathologic conditions. Monocyte chemokine-activating peptide (MCP-1), a member of the C5a subfamily, has been shown to have a potent chemotactic activity for monocytes in vitro and to be expressed during the development of experimental immune encephalomyelitis, an animal model of multiple sclerosis. In order to study the biological role of MCP-1 in vivo, and to examine its role in the development of brain inflammation, we generated transgenic mice in which MCP-1 expression is driven by the myelin basic protein promoter. In these transgenic mice, we observed maximal transgene expression (RNA) at three weeks of age throughout the white matter, as expected for this oligo-astrocytic promoter. Coincident with the temporal and spatial pattern of transgene expression, we found a significant infiltration of mononuclear cells. The recruited cells were predominantly macrophages, as determined by histochemical and immunohistochemical criteria.

These results clearly demonstrate that MCP-1 can direct monocyte trafficking in vivo. Furthermore, they establish these transgenic mice as important models to study inflammatory cell recruitment to the CNS and the involvement of chemokines in the etiopathology of inflammatory brain disease.


Many proinflammatory complement (C) components are associated with amyloid deposits in Alzheimer disease (AD) brain. However, the role of C5 in AD is little understood and may be pertinent to the recruitment and activation of inflammatory cells. Because astrocytes express C5a receptor (C5aR) and because of the C5a derived anaphylatoxin C5a control of cytokine expression in peripheral cells, we further explored the secretion of TNF and IL-6 in astrocytes of C5 deficient (C5-/) mice and compared with astrocytes from congenic C5 sufficient (C5+/-) mice. Transgenic mice overexpressing C5a and control C5-/--mice were sacrificed at 12 weeks of age. The presence of C5 led to significantly increased levels of IL-1alpha and TNF-alpha in C5+/- mice. Altogether, these data suggest that glial cells derived from C5 deficient mice are either deficient in some cholinergic trophic agent or, more probably, produce some material with a negative effect on neuronal function with some specificity for cholinergic neurones.

828.6 GENOMIC IMPRINTING AND AUDIOPHONIC SEIZURES IN MICE. M. Banks, K. Allen, B. DePina, P. Neumann and T. Gardner*.

Audiogenic seizures (AGS) are severe convulsions induced in mice by loud, high-frequency sound. AGS susceptibility in DBA/2 (D2) mice is maximum at 21 days of age and gradually subsides with adulthood. Epilepsy prone (EP) mice were selected for high AGS susceptibility. Unlike D2 mice, EP mice are maximally AGS susceptible at 30 days of age, and this susceptibility persists into adult ages. The seizure phenotype is similar in both the EP and D2 mice. Crosses were generated between the EP and D2 mice to determine the genetic mechanism that may account for the differences in seizure susceptibility at 30 days of age. Although EP and D2 mice exhibit high susceptibility to AGS at 30 days of age (66% and 58% seizure, respectively), the reciprocal EPD2F1 and D2EPF1 hybrids were more seizure resistant, (14% and 16% seizure, respectively), suggesting that AGS susceptibility is a traits controlled by genes in a recessive trait. In the EP EPD2F1 backcross, significant associations were found between AGS susceptibility and several markers near the ras gene on mouse 11. In the reciprocal D2EPF1 X EP backcross, however, no significant associations were found between AGS susceptibility and these markers. These data suggest that genomic imprinting may influence the expression of genes on chromosome 7 involved with AGS susceptibility. Moreover, these findings indicate that different genes are responsible for AGS susceptibility in the EP and D2 mice, further substantiating interallelic heterogeneity for audiogenic seizure susceptibility in mice. (Supported by NIH grant N22365, Boston College REU and MCR-Canada.)
828.7 PROGRESSIVE COGNITIVE IMPAIRMENT IN DISCRIMINATED ABDUCTION PERFORMANCE IN GFAP-IL6 TRANSGENIC MICE. J.H. Bostick, J.C. Bost, R.A. Lipton, I.L. Bost, Dept. of Neuropsychiatry, The Scripps Research Institute, La Jolla, CA 92037.

Transgenic mice that express the pro-inflammatory cytokine IL-6 in the CNS under the regulatory control of the glial fibrillary acidic protein promoter (GFAP) develop progressive neurodegenerative disease. The relationship between the neurological disease progress and functional neurobehavioral outcomes was examined. Heterozygous or homozygous control mice and control breeds were repeated at different ages in a Y-maze for their ability to learn a nonconditional spatial discrimination to avoid the onset of a mild foot shock. The extent of the ability to learn the task and errors were used as an index of learning performance. When tested at 3 months of age, heterozygous mice were at the same rate of control mice, whereas homozygous mice were significantly slower to acquire the avoidance response. At 6 months of age, heterozygous mice exhibited a deficit in learning the avoidance response intermediate to that of the controls and homozygotes. At 12 months of age the performance of both heterozygous and homozygous mice had declined even further. At this age both groups failed to demonstrate acquisition of the avoidance response and made significantly more errors when compared to age-matched controls and their own performance at 3 and 6 months of age. No correlation was observed between motor function/coordination and the impaired learning ability of the transgenic groups and no differences were observed between the groups in a test of shock sensitivity. These data demonstrate a progressive learning disability in the GFAP-IL6 mice which is correlated with the progressive neuropathological changes observed in these animals and is not consequent to impaired motor or somatosensory function.


Under physiological conditions, the dopamine transporter (DAT), located at the plasma membrane, is responsible for the uptake of dopamine (DA) from the synaptic cleft back into the cytoplasm, where it may be repackaged by the vesicular transporter (VAT) into storage granules, enabling further release of DA from the synaptic cleft. Several studies have shown that DAT transporters play an important role in neurotransmission and could be targets for pathological and/or pharmacological agents. Although the mechanisms involved in dopaminergic cell loss in the homozygous weaver mouse (w/w) are still not fully understood, the earliest and largest detectable decrease in striatal DA uptake (Simon et al., J. Neurochem. 52,455,1994). The cause of this decrease remains unclear but it could be a consequence of the decrease in DA expression of uptake of/ and/or storage-related proteins induced by the weaver mutation. Quantitative in situ hybridization (ISH) techniques show that DAT and DA transporter (TH) mRNA, carried out on surviving DA cells of the substantia nigra pars compacta (SNpc) from two mouse strains with a significant decrease of DAT expression (μ<0.05) when compared to normal control mice (μ<0.0/). DAT mRNA also decreases in SNc cells in heterozygous weaver mice (w/a) although differences are not significant. In contrast the expression of VAT and TH mRNA was not affected in w/a mice compared to DAT mRNA of the weaver mouse (w/w). The specific decrease of DAT mRNA in the surviving neurons of the adult weaver mouse could result from a feed-back regulation due to a deficient interaction between dopaminergic nerve terminals and striatal targets. Nevertheless, a genetic alteration of DAT can not be excluded. Studies of DAT expression at early stages during post-natal development are now underway to address these questions.


Several filial generations after a C57BL/6 × BALB/c cross, a single female mouse in a litter of five was noted at 43 days of age (P43) to show weakness of hindlimbs, tremor, and instability of gait. The motor disorder progressed, and at 6 months of age, weight and gait variability similarly affected mice of both sexes were produced by her sisters, and in subsequent generations in this pedigree. Affected mice have not generated offspring, but the 23/109 affected progeny were homozygous. The phenotype strongly suggests autosomal recessive inheritance. At autopsy, the liver color is pink to yellowish-white. Many liver cells are enlarged with multiple vacuoles and granules, some staining with Sudan black B for lipids and with the fluorescent Filipin reagent for unesterified cholesterol. Macrophages rich in cytoplasmic granules are prominent in spleen, lymph nodes, and lung. Large cells in bone marrow smears showed intense cytoplasmic fluorescence with Filipin. Staining of paraffin sections of formalin-fixed tissues with the periodic acid-Schiff method is variable from cell to cell in many organs, but is never strong. Brain weight was abnormal and densities of large neurons in dorsal root ganglia, spinal cord, cerebellum, cerebral cortex and retina were dis tended with lysosome-sized granules that vary in EM appearance from membrane-bound homogeneous organelles to granules rich in myelin-like membranes. Many myelinated and unmymelinated axons in CNS and PNS are dramatically distended focally to 5-10 times their normal diameter with similar granular inclusions. Cerebellar Purkinje cells are reduced in number. These features are consistent with descriptions by others of mouse and human inherited disorders(s) classified as Niemann-Pick Type C disease. Chemical, genetic and tissue culture tests are in progress.


The role of long term potentiation (LTP) has been hypothesized to be important in the kindling model of epileptogenesis. The role of long term depression (LTD) in kindling has been more controversial. The availability of a line of transgenic mouse with a mutant form of calcium calmodulin-dependent protein kinase II, resulting in the absence in LTD in response to 5-10 Hz stimulation (Mayford et al., submitted), allows an examination of the role of LTD/LTP in epileptogenesis. Therefore we surgically implanted indwelling electrodes into the amygdala and applied a 1 sec train of 5-10 Hz stimuli once daily to evoke after-discharge (AD). The AD is a prominent fraction of which is the 5-10 Hz range. The duration of evoked AD was nearly twice as long in mutants as in wild-type mice in both the stimulated and contralateral amygdala.Despite this, the rate of kindling to the first generalized convulsion did not differ between the groups (mutants = 120 ADs, wild-type = 109 ADs). The kindled state was maintained equally well by the two groups and required stimulation-free intervals. These indicate a partial dissociation between AD duration and rate of kindling development. This suggests that a reduction in LTP associated with epileptogenic spikes in the range of 5-10 Hz may be linked to the failure of the mutant to develop kindled seizures more rapidly than wild-type mice. Supported by NSERC and HHMI.


Successful gene targeting by homologous recombination is largely determined by the frequency of homologous vs. nonhomologous recombination events. A widely used selection method for homologously recombinant ES cells is a positive-negative selection (PNS) which typically involves the co-introduction of a selectable marker gene (pcd) together with an essential gene (D2) under control of the D3 receptor (Tk) gene outside of the targeted homology and the addition of antiviral drugs to the ES cell-selecion media. However, the toxicity of such drugs (gancyclovir or FIAU) is thought to impair the efficiency of ES cells to colonize the germ line. An alternative to the use of antiviral agents in conjunction with G-418 for PNS is to destabilize only that portion of the neomycin phosphotransferase (Neo) gene, and that derives from random integration sites (positive selection). We have targeted the dopamine D3-receptor gene locus in mouse ES cells with 5 different replacement vectors. Four vectors contained only one of the following sequence elements that are thought to destabilize the neomycin-resistance-gene (neo) transcript: 1) a poly (A) tail less neo-cassette; 2) a neo ribosome with proven catalytic activity, 3) a mutant ribosome placed outside of the neomycin-resistance gene (control); and 4) an antisense neo sequence. A control vector contained the TK gene placed outside of the targeted homology. Our initial results indicate that these strategies are effective at enriching the selection with antiviral agents. (Supported by NSF IBN-9409772).
828.13 GENE TRANSFER AND THE EXPRESSION OF AN EXOGENOUS GENIE IN VIVO USING THE HUMAN TUMOR VIRUS OF JAH-1-LIPOSOME METHOD. K. Kato, A.B., and S. E. Fasa
dNara Institute of Science and Technology, 891-65 Matsukawa, Ibaraki, Japan. We have established a simple and efficient method for gene transfer in vivo in post-mitotic neurons of adult rat brain using nuclear protein, liposomes, and a recombinant virus of the human genome. Simple plasmid DNA and nuclear protein (HMG1: non-histone chromosomal protein high mobility group 1) that coexists in vivo were co-introduced into cells by HVJ-mediated fusion. The DNA was then carried into the nuclei of newborn cells with the aid of the nuclear transport system of HVJ. HVJ-liposomes containing the E. coli β-galactosidase gene under control of a chicken parvalbumin promoter and retroviral vector of E. coli were injected into various brain areas of adult rats, such as hippocampus, cerebellum, cerebral cortex, or caudate putamen. Neuronal cells that expressed the β-galactosidase gene were detected only in the target area of the injection and not in adult rat for 10 days by light microscopic analysis. Electron microscopic analysis revealed that the protein of the histocompatibility was associated with the nuclear membrane and the endoplasmic reticulum of positive cells; it appeared that the products were translated endogenously. Moreover, the enzymes were observed in typical cytoplasmic granules, round, and pale nuclei, and with direct axo-somatic and axo-dendritic synaptic contacts. We are currently applying this system to investigate morphometric development in the chicken embryo hindbrain.

828.15 CYTOCHROME OXIDASE ACTIVITY IN THE NAPLES HIGH- AND LOW-EXCITABILITY RAT LINES: A STUDY OF BRAIN ACTIVITY DISTRIBUTIONS IN A GENETIC MODEL. K. Niwa, D. Hu, E. Gonzalez-Lima, and A.G. Sadile1 Dept. of Psychology and Inst. for Neuroscience, University of Texas at Austin, Austin, TX, 78712, USA. 1Inst. of Human Physiol., 1st Medical School, University of Naples, Italy. Quantitative Cytochrome Oxidase (C.O.) histochemistry, a metabolic marker for neural activity, was used here, for the first time, to detect functional brain differences between the three different genotypes. The Naples High-Excitability (NHE) and Naples Low-Excitability (NLE) rat lines were selectively bred according to the frequency of recurrent seizures during forced exposure to spatial novelty, both of which are hallmarks of hippocampal dysfunction. Although several neuro-behavioral studies have shown these animals to be impaired on a model for hippocampal function, these results need to be confirmed by other means. Using quantitative image analysis, six areas of interest within the hippocampal formation, including the CA1, the CA3, the dentate gyrus, and the outer blade of the dentate gyrus, were sampled in naïve rats. Significant differences in C.O. activity were detected between the NHE and NLE rats models for hippocampal function, and, perhaps, a generalized genetic model of hyperactivity.

828.16 ASSESSMENT OF THYMIDINE KINASE DEFICIENT HERPES SIMPLEX VIRUS TYPE 1 AS A TRANSFER VEHICLE INTO SYMPATHETIC PREGANGLIONIC NEURONS OF THE DOG. J.G. Gonzalez-Lima, S. Kato, E. Kato, and H. Shimizu. National Institute of Neuroscience, Research Group, Roberts Research Institute, London, Ontario, N6A 5K9. The sympathetic nervous system discreetly controls blood pressure as well as blood flow to different viscera and organs. Long term improvements in faulty control of discrete functional groups of spinal sympathetic preganglionic neurons (SPN's) might be accomplished by introducing overexpression in these cells using replication deficient herpetic simplex virus type 1 (HSV-1). Our initial experiments assessed the suitability of HSV-1 as a transfer vehicle into SPN's. The Escherichia coli β-galactosidase (β-gal) gene or the human pheochromocytoma phosphatase (PPH) gene was inserted into the thymidine kinase gene, generating replication-defective TK-β-gal or TK-AP (thymidine kinase mutant of HSV-1 expressing β-gal or AP, respectively). To assess whether nerve-specific promoters could be inserted into HSV-1, the TK-β-gal was directly incoated into the spinal cord of hamsters at thamic T (segment 5-6). At 3 or 5 days post-inoculation, neurons and numerous oligodendrocytes in the spinal cord were infected with TK-β-gal. To target only SPN's, we used an uptake and retrograde transport of this replication defective HSV-1 into the cord, the TK-AP was inoculated into the lateral adrenal gland of hamsters. Two, four or five days later, the spinal cords were removed and examined for the presence of TK-AP using AP histochemistry. At 2 days, many SPN's expressing AP were found in T9-8. Very few oligodendrocytes were infected and few inflammatory infiltrates were visible upon counterstaining the cord. At 4 days, fewer SPN's expressing AP were detectable and infiltrates were present. At 5 days, very few SPN's expressing AP were found and inflammatory infiltrates were more abundant. In more preliminary experiments TK-β-gal also was retrogradely transported from the adrenal gland into the spinal cord. These studies demonstrate that a TK-HSV-1 can be retrogradely transported from the periphery to the spinal cord to transduce target groups of SPN's specifically supported by MRC Canada.

DEVELOPMENTAL DISORDERS III

829.1 QUANTIFICATION AND SPECIFICITY OF HIPPOCAMPAL NEURAL LOSS FOLLOWING NEONATAL INFECTION WITH LCMV. B.D. Passare, A.C. Bishop, J.T. Hedlund, M.J. Hoenicka, M.J. Bachmayer, R. Baldessar, A.J. Miller Emory Univ Med, Atlanta, GA 30322. Scipps Res Inst, La Jolla, CA 92037 While a perinatal viral insult has been implicated as a potential causative factor in developmentally-derived hippocampal pathology, the mechanism by which a viral infection during development could disrupt hippocampal structure and function has not been defined. Accordingly, we infected neonatal rats i.e. with lymphocytic choriomeningitis virus (LCMV) and measured electrophysiological and neuropathological changes in the hippocampus. In rats studied at 84-102 days post infection, virus was cleared from dentate granule neurons, yet, Nissl stained cells in the dentate granule layer were decreased by 67.5% (internal limb) and 74.9% (external limb) in LCMV-infected rats (p<0.002). In addition, in vivo electrophysiological measures, recorded from the hippocampal dentate granule layer in response to paired-pulse stimulation of monosynaptic inputs, revealed a marked decrement in the OAB-mediated recurrent inhibition to dentate granule cells, suggesting that dentate granule cells were receiving enhanced excitatory input, perhaps due to an early loss of GABAergic inhibitory interneurons. A subset of GABAergic interneurons in the dentate gyrus contain parvalbumin which may protect these cells from Ca2+-induced damage. Quantification of interneurons stained for parvalbumin revealed a 72% loss of these cells in the dentate gyrus of LCMV infected rats (p<0.05). Since rats were infected prior to the developmental stage at which parvalbumin appears, immature neurons (those lacking their protective parvalbumin expression) are likely to be directly affected by the effects of the virus. These data suggest a novel neuroprotective mechanism involving an early virus-induced loss of inhibitory interneurons resulting in an unshackling of excitotoxic synapses on dentate granule cells (with resultant cell death), and thus perpetuation of a pathologic cascade which continues in the absence of detectable virus.

829.2 EFFECT OF CHRONIC HYPOXIA ON CORTICAL CELL NUMBERS AND VASCULAR DENSITY. W.B. Siegel*, M.A. Salazar, V.P. Pathy, G.G. Hedden, J.R. Ment, and M.L. Schwartz. Dept of Surgery (Anatomy), Pediatrics, Neurology and Neurobiology. Yale Univ. Sch. of Med. New Haven CT 06517. Premature infants often undergo chronic hypoxic episodes. In order to examine the effects of hypoxia on brain development, we have examined the brains of rats 1 to 33 in a chamber where O2 levels were controlled at 9% to 10%. The rats were sacrificed and their brains removed for a quantitative study of cells and microvessels of the neocortex. Cell counts were performed on Nissl stained sections using the optical dissector method. Neurons and glial were counted separately, using nuclear morphology to assign them provisionally to either category. Microvessels were counted using the dissector method in unainted, unstained, randomly oriented cortical sections. Hypoxic rats (N=5) had smaller cortical volumes than control rats (N=5) (217 mm3 vs 156 mm3, p<0.03). Also, the density of neuronal-like cells was higher in the hypoxic rats (124,000/mm3 vs 96,000/mm3, p<0.01). Despite the smaller cortical volume, there were more of these cells in hypoxic than controls (22.2 million/mm3 vs 14.9 million, p<0.005). Coincidentally, the number of cells containing some Prussian blue stainability (specific for Siderotic iron) was similar (2.6 million hypoxic vs 2.2 million control). Hypoxic rats had a higher density of capillaries than controls (518 cap/mm2 vs 400 cap/mm2, p<0.03). We conclude that neonatal brain has a high number of adaptive responses to hypoxia. These include increased vascular density and alterations in programmed cell death. Definitive analysis of this neonatal mechanism is currently being examined using immunohistochemical methods to discriminate between neurons and glia. In addition, these changes in cortical volume and cell density suggest that there may also be decreases in the volume of the neuropil or the extracellular space. This work was supported by N35278.
829.3
PRENATAL γIRRADIATION REDUCES PREPULSE INHIBITION OF STARTLE RESPONSE IN RATS. M. Mintz, A. Glog, R. Berthelot, M. Macri. Department of Psychology, Tel Aviv Univ., Ramat Aviv, Israel.

The pathophysiology of behavioral anomalies caused by prenatal irradiation is frequently obscured by the complexity of behavior used in such studies. To simplify the experimental models we explored changes in the startle response: PPI in Sprague Dawley rats submitted to whole body γ irradiation (Theratron 780 CG/C source at 1.5 Gy and a dose rate of 0.15 Gy/min) on Days 15, 17, or 19 of gestation (G). Sham-irradiated rats served as controls. At 25 days (P25), startle response was assessed to a train of white-noise bursts (122 dB, 40 msec). Following habituation (30 trials), rats were exposed to a white-noise pre-pulse (20 dB: 150-100 msec). At P25 all irradiated groups had higher startle response to the first noise burst compared to controls (F3,35 = 4.1, p<0.02). In G15, startle amplitude remained elevated throughout the exploration. By contrast, other irradiated groups habituated to control level at the same rate. PPI was reduced in all irradiated groups. The onset of startle at P55, showed no facilitation in G17 and G19 groups. In G19, habituation to control levels was markedly delayed (t10 = 6.8, p<0.01). PPI remained decreased in both irradiated groups (animals irradiated on day 15 were not available for analysis) but not at statistical significance level. The possibility is discussed that reduced PPI is associated with deficient telencephalic structures, perhaps the prefrontal cortex and hippocampi that are implicated in PPI of startle response.

829.5
NEUROANATOMICAL ANOMALIES IN MICE EXPOSED TO PRENATAL RADIATION. S. Soong, S. Schmidt*, M. H. Fouse (1), and Y. Amuso-Vilaca (1). (1)Dpt. of physiology, Universidade de Sao Paulo, R. do Jardim, R.J. 20550120, Brazil, 2(Dpt. Psychology, University of Alberta, Canada.

In adult mice, prenatal x-irradiation at 17, 19 and 17 days of gestation with 30 Gy causes reduction of the corpus callosum (CC). Here, we reported the short term effects after irradiation at E16 with 30y and the effects in adult animals after irradiation at E15 with doses lower than 3 Gy (0.5, 1.5, 1.75, 2, 2.5, 3 Gy). To study the short-term effects, offspring of control and irradiated mothers were observed at E17, E19, PND1 and PND3. The brains were sectioned and stained with either cresyl-violet or Bodian silver-stain. The brains of animals irradiated at E15 were cut out and stained with cresyl-violet. In normal E17 mice, some cells in the subventricular zone formed a slug across the midline. One day after irradiation, the subventricular zone was atrophied, the slug was abolished, and a great number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the zona incerta). At PND1, the cells in the thalamus were reduced and a number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the zona incerta). At PND1, the cells in the thalamus were reduced and a number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the zona incerta). At PND1, the cells in the thalamus were reduced and a number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the zona incerta). At PND1, the cells in the thalamus were reduced and a number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the zona incerta).

829.7
MEDIAL SEPTAL ATROPHY IN AGED MICE EXPOSED TO A SINGLE DOSE OF ALCOHOL IN UTERO. J. Lee, R. Dumais, M.H. Lee and A. Rabe*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

It has been demonstrated that mice exposed to a single dose of alcohol during neurotransmission, while still remaining free of gross malformations, a precipitation of abnormal cell loss in middle and old age. In order to determine whether the behavioral deterioration is related to anatomical changes in specific brain structures, we performed morphometric analyses in several forebrain structures.

C57Bl/6j mice were exposed to ethanol on gestation day 15 (GD 15) to a single oral dose of 5.8 g/kg alcohol (E), which produced approximately 700 mg/dl BAC 1 hour after gavage. Control mice were intubated with isocaloric and isovolumic dextrose solution substituted for alcohol (D). The brains were sampled at 24 or 32 months post-exposure. The number of neurons was counted and neuron size was measured in the occipital cortical layers, hippocampus, and medial septum. The only measure yielding a significant group difference was the neuron size in the medial septum. They were significantly smaller in the E than D mice. The diminished neuron size is consistent with the findings that structures along the midline are particularly susceptible to alcohol exposure on GD 9. The involvement of the medial septum in long-term memory has been documented, and the atrophic medial septal neurons in the E mice may account for the premature memory decline reported previously by our laboratory (Dumas and Rabe, 1994).

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Epilepsy: Kindling

829.1
AMYGDALA-KINDLING LEADS TO LOSS OF GABA-IMMUNOREACTIVE NEURONS IN A TINY AREA OF THE PIRIFORM CORTEX. H. Lehmans, U. Brent and W. Lüth, (SPP: European Neuroscience Association, Dept. of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, D-30559 Hannover, Germany.

Female Wistar rats weighing 250-280 g were electrically kindled via bipolar electrodes, which were implanted unilaterally into the basolateral amygdala, until 10 fully kindled seizures were elicited. After an unstimulated, right-hand period of 40 days the rats were sacrificed and an immunohistochemical preparation of brain tissue was performed by using a monoclonal anti-GABA antibody. Quantification of GABA-immunoreactive (IR) neurons was carried out in the basolateral amygdala, piriform cortex, dentate gyrus, CA1 and CA3. A kindling (n=10) versus a sham-group (n=6), which received electrical implantation, but no electrical stimulation, and a naive group (n=10) were compared. At the stimulation site i.e. the nucleus of the right basolateral amygdala, a significant loss of GABA-IR neurons was observed in kindled rats that ranged between 31-52%. The extent of cell loss was similar ipsi- and contralateral to the stimulation site. Along the rostro-caudal extension of the piriform cortex a distinct area between +0.2 and -0.8 mm from bregma to the stimulation site showed a significant decrease in GABA-IR cells by 17-20%. In contrast, the hippocampal formation showed no changes in GABA-immunoreactivity. In the sham-group no alterations in any of the above areas were evident. We conclude that amygdala-kindling is associated with permanent GABAergic cell loss in the site of stimulation, but also at a circumscribed site of the piriform cortex, which is evidently involved into the phenomenon of kindling.

Supported by the Deutsche Forschungsgemeinschaft (La274/5-1)

829.2
PERSISTENT SPONTANEOUS EPILEPTIFORM DISCHARGES EVOKED BY KINDLING-LIKE STIMULATION IN PIRIFORM CORTEX IN VITRO. M. R. Pulitzer and P. L. Carney, (NIMH, National Institute of Mental Health, Bethesda, MD 20892, USA). We have reported that rats kindled in vivo show spontaneous epileptiform discharges during hippocampal slices as well as during kindling stimulation.

The present study was designed to investigate the mechanisms of kindling-like stimulation in the piriform cortex and to compare the effects of kindling-like and kindling stimulation. The piriform cortex was selected for study because of the close relationship between the piriform cortex and the hippocampus, and because of the similarity of the kindling-like and kindling phenomena in the two brain regions.

The piriform cortex was lesioned by kindling-like stimulation in vivo (kindling stimulus) or in vitro (kindling-like stimulus). Kindling-like stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex. Kindling stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex. Kindling-like stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex. Kindling stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex. Kindling-like stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex. Kindling stimulation was performed by applying a constant current of 0.1 mA for 10 min to the piriform cortex.

The results of our study indicate that kindling-like stimulation in the piriform cortex induces a persistent epileptiform discharge in the hippocampus. These results suggest that kindling-like stimulation in the piriform cortex may be a useful model for the study of kindling and kindling-like phenomena in the hippocampus.

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830.3


"Wet Dog Shakes" (WDSs) are associated with kindling of the septum, hippocampus (HC), entorhinal cortex (ERC) or amygdala (Quillacalla, et al., 1980; Le Gal La Salle et al., 1981; Lerner-Natoli et al., 1984). Injection of colchicine into the HC suppressed by 95% the WDS elicited via ERC kindling (Fruth & McNamara, 1986). We evaluated the effect of ibotenic acid (IA) lesions of the HC on WDS elicited by amygdaloid kindling. Bilateral stereotaxic injections of IA into the HC were performed in anesthetized male SD rats and chronic amygdaloid electrodes were placed (Meyerhoff, et al. 1985). IA lesions destroy neurons without affecting fibers of passage. Although lesions did not affect no. of stimulations required to elicit stage 5 seizures, the no. of WDS observed was reduced by 68%. Injection of (TRH) i.e. synchronizes H EEG (Kalivas, 1980) and elicits WDS (Drust & Crawford, 1983). Although other manipulations might be involved in WDS, kindled seizures increase brain levels of TRH (Meyerhoff, et al. 1990) and HC levels of TRH mRNA (Rosen et.al., 1992; Kubek et al., 1993).

830.5

THE EFFECTS OF Picrotoxin and Atropine on Interictal Spiking During Amygdaloid Kindling in the Genetically FAST and Slow Kindling Rat Strains. J.A. Grabowski and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

In this study, two rat strains with naturally different kindling rates and disposition to interictal spiking (ISIs) were examined. Although IIS is the hallmark of an epileptic focus, the kindling resistant (SLOW) rats showed greater ISIs to a greater number of stimulations during the stages of amygdaloid kindling than the kindling prone (FAST) rats. In the present study, we examined the ISIs, the convulsive seizure (ictal event) and the postictal spike (PIS) for their pharmacological sensitivity in both the FAST and SLOW kindling strains. Rats were amygdaloid kindled daily at their afterdischarge threshold intensity. Once IIS was established, rats were injected with either drug or saline in a latin square design and the IIS rate was observed for 20 min. Subsequently, the ictal event was triggered, which provoked PISs. The GABA antagonist, picrotoxin (0.5 mg/kg), significantly increased the IIS rate, yet had little effect on the ictal event in both strains, while it slightly depressed the PIS rate in the SLOW rats. Like picrotoxin, the cholinergic antagonist, atropine (25 mg/kg), increased the IIS rate in both strains, but strongly suppressed the ictal event in the SLOW but not the FAST strain. Clearly, both IIS activity and kindling rate show a genetic predisposition, but surprisingly their relationship is inverse. It is also clear that IIS, ictal and PIS events are neither similar in the two strains nor in their sensitivity to pharmacological manipulation.

830.7

AMYGDALA KINDLING PRODUCES MORE "DEFENSIVE" BEHAVIOR THAN HIPPOCAMPAL OR CAUDATE KINDLING IN RATS. J.P. Cantin, E.L. Kalynchuk, and D.P. McNamara. Departments of Psychology and Neurology, University of British Columbia, Vancouver, B.C., V6T 1Z4 and Department of Psychology, Univ. of Alberta, Edmonton, Alberta, T6G 2E9.

We have previously reported that amygdala-kindling in rats results in elevated levels of several defensive behaviors (Kalynchuk et al., 1995). The purpose of this experiment was to determine whether this effect would also occur after kindling different brain sites. Bilateral electrodes were implanted in the basolateral amygdala, entorhinal cortex, hippocampus, or caudate nucleus of 60 male, Long-Evans rats. These rats were divided into six groups: amygdala-kindled, amygdala control, hippocampal-kindled, hippocampal control, caudate-kindled, and caudate control. All rats then received 99 convulsive (kindled) or sham (control) stimulations. One day after its last stimulation, each rat was placed in an open field for 5 minutes and then tested for its resistance to capture from the open field. The following day, each rat was tested on an elevated-plus maze. The site of stimulation had a significant effect on the results of these tests. During the first minute in the open field, the amygdala-kindled rats engaged in less exploration than both the hippocampal or caudate-kindled rats. In addition, the amygdala-kindled rats were more resistant to capture from the open field than both the hippocampus or caudate-kindled rats. Finally, the amygdala-kindled rats engaged in more open arm activity and escape behavior post-stimulation relative to the hippocampal or caudate-kindled rats. Thus, amygdala-kindling results in increased emotional reactivity and defensiveness compared to hippocampal or caudate kindling. This suggests that heightened emotional intensity in kindled rats may be a consequence of repeated seizure activity in or near the amygdala, rather than the kindled state per se. These findings may be relevant in understanding the fact that affective disturbances often experienced by temporal lobe epileptics. (Supported by NSERC grants to D.T. and J.P.J.P., and an M.R.C. scholarship to L.E.K.)

830.8

HIPPOCAMPAL KINDLED SEIZURES DISRUPT PERFORMANCE IN THE MORRIS WATER MAZE. T.H. Gilbert* and M. Corcoran. Dept. of Psychology, Univ. of Victoria, POB 1050, Victoria, BC, Canada V8W 2Y2.

The hippocampus is thought to play an important role in the processing of spatial information, in that interference in normal hippocampal functioning can produce deficits in performance that use spatial navigation for optimal performance. The effects of hippocampal kindling on subsequent learning and memory of a spatial task have been assessed in several studies. Although the studies vary in procedures and methods, results fairly consistently suggest that performance activity within the hippocampal formation impairs performance. We have attempted to assess the spatial performance of rats in the Morris water maze task during kindling of hippocampal field CA1.

We used two procedures: (1) seizures were kindled with stimulation of CA1 prior to daily training in the water maze (acquisition); and (2) maze training was imposed until performance was disrupted and the seizures were kindled with suppression of CA1 prior to daily testing in the maze (retention). In both cases, stimulation of field CA1 was applied 25-45 min prior to daily testing in the maze. Only convulsive afterdischarges were kindled and those that occurred in the experimental group, whereas in other rats generalized convulsive seizures were kindled. Yoked controls carried electrodes but did not receive stimulation.

We found that CA1 kindled seizures significantly disrupted water maze performance during both acquisition and retention. Similar effects were produced when either nonconvulsive seizures (AD) or convulsive seizures were evoked. Our findings are consistent with previous results suggesting that kindling epilepsy activity in the hippocampus disrupts spatial learning and memory. (Supported by NSERC)
EPILEPSY: Kindling, an animal model of epilepsy, was investigated for having acute and chronic effects on memory. Genetically FAST and SLOW kindling rat strains were used for working and reference memory in a T-maze before, during, and after kindling. Maze performance was monitored 23 hours after eliciting stage I through 5 kindled convulsions, then 1 hour versus 10 minutes after stage 5 convulsions in fully kindled animals. After a seizure-free period of 10 days and beyond, memory performance was retested with increasing levels of difficulty in the working memory task. Results indicated that kindled convulsions and kindling per se, have acute and chronic effects, respectively, on working memory performance. Working memory was more susceptible to disruption than reference memory. Additionally, the FAST rats were less capable than the SLOW rats on the working memory task before kindling, and showed greater impairment during and after kindling. Discussion focused on methodological differences between limbic areas of FAST and SLOW rat strains that may account for the differential sensitivity of working memory to kindling disruption.

Regional Increases in the 2 subunits but not of the 1 subunit of calmodulin Kinase II mRNA in Kindling. K. Sato*, K. Kitashara*, K. Muramori*, and T. Hayakawa*. Department of Psychiatry and Department of Neurosurgery, Okayama University Medical School, Okayama 700, Japan. Clinical Research Institute, National Satsunai Minamiyama Hospital, Okayama 701 03, Japan. Department of Neurosurgery, Kagawa Medical School, Kagawa 761 07, Japan.

Levels of the mRNAs for the α and β subunits of calmodulin CK II in rats were studied using a hybridization model of epilepsy. Using a highly hybridized 35S-labeled oligonucleotide probe, induction of these mRNAs was evaluated in the rat brain just after and at 0.5, 1, 2, 4, 8 and 24 h after generalized seizures induced by daily applications of the same dose (0.5 mg/kg), for 4 to 24 h after the seizures. Kindling significantly increased levels of the β subunit of Calmodulin Kinase II mRNA in the hippocampus compared with controls which had undergone a sham procedure. This increase occurred 24 h after the last application of the β subunit of Calmodulin Kinase II mRNA significantly increased by 18 to 28% in the granule cell layer on each of the dentate gyrus and by 18 to 30% in the pyramidal cell layer of the CA3. In the pyramidal cell layer of the CA1 region, levels increased significantly by 22% 4 h after the seizures. There were no detectable changes in levels of the β subunit of the episode mRNA in other regions, which included the amygdala, piriform cortex, perirhinal cortex and temporal cortex. In contrast, no significant changes in levels of the α subunit of Calmodulin Kinase II mRNA were observed in the regions examined after kindling induced generalized seizures.

These results indicate that the increases in CaM kinase II dependent protein phosphorylation may be associated with changes in synaptic biochemistry in kindling, and suggest that CaM II kinase may mediate the molecular processes underlying kindling induced epileptogenesis.


Prior work has shown that an antecedent tone presentation during every kindling trial significantly delays the rate of amygdala kindling (Hernandez. Winer, Klein, & Kahler, 1995). The specific aim of this study was, therefore, to alter the presence of the tone and investigate this manipulation on kindled seizure development. To achieve this goal, male Long-Evans rats were implanted with a right amygdala and assigned to either a Tone, No Tone, or Tone Discontinued group and kindled daily. Each tone on every trial while the Tone Discontinued group received it for only the first 5 days and subsequently kindled the same as the No Tone group (i.e., not exposed to the tone while receiving the kindling stimulation). The results revealed that prior exposure of the tone significantly delayed Stage 5 seizure development for subjects kindled in the central nucleus and significantly accelerated Stage 5 seizures for those kindled in the amygdalohippocampal transition area; this finding was observed whether the tone was presented at each trial or discontinued early during the kindling process. These findings indicate that an auditory stimulus presented prior to amygdala kindling alters the rate of seizure progression. Furthermore, the alteration is not contingent on continuous exposure to the tone, but does appear to be region-specific. Lastly, the data suggest there is an early critical period during kindling that may be amenable to manipulations. Further research is necessary to determine the nature of these manipulations and what effects they might have on seizure genesis.

Supported by NIH Grant No. NS20555 and the Alfred P. Sloan Foundation (T.D.H.), an APA Minority Neuroscience Fellowship (A.E.K.), and the University of Colorado (V.R.).

SEIZURE AND SPIKE ACTIVITY INTERRELATIONS DURING RAPID KINDLING IN RABBITS. Oleg A. Timofeev* and Gary M. Peterson. Dept Anatomy & Cell Biology, East Carolina University, Greenville, NC 27858.

Seizures and interictal spikes are considered to be basic components of epileptic activity, but the relationship between these is poorly understood. The present study examines the relationship between seizure and spike activity using closely-spaced recurrent hippocampal seizures ("rapid kindling"). This model provides an opportunity for examining electrophysiological responses in response to electrical stimuli applied at different stages of seizure susceptibility. In a group of 8 rabbits with chronically implanted electrodes were stimulated in the hippocampus once every 5 min to achieve a condition of rapidly recurring seizures. EEG was monitored continuously during the experiment (3.6 h). Electrical stimulations produced progressive seizure development in 8 of the 12 animals. Seizures were induced more frequently with increasing numbers of electrical stimulations. Interictal spiking was observed in 5 of the 8 rabbits (4-4.5 h) which did not develop seizures. Spiking developed at different stages of kindling. A latency for spike onset following each electrical stimulus train significantly fluctuated due to changes in seizure susceptibility. Two kinds of spike onset latency were distinguished: "short-term" (< 50 s) and "long-term" (> 70 s). "Short-term" spike latency was observed predominantly in 3 situations: in the early phase of a prolonged refractory period (5 min after triggering seizure), when seizure decay occurred, and during an "unsuccessful" kindling procedure. "Long-term" spike latency was observed predominantly in the last phase of a prolonged refractory period (5 min before triggering seizure) and always during the development of ictal events. These data showed that "short-term" latency for spike onset correlated with low susceptibility to seizure whereas "long-term" latency correlated with enhancement of seizure susceptibility. These results suggest that seizure priming and triggering mechanisms temporally inhibit spike activity.

DIFFERENTIAL EXPRESSION OF A AND GLUTAMATE TRANSPORTER SUBTYPES IN FULLY KINDLED ANIMALS. T.D. Hernandez, M. Nakashima, H. H. Parry, and N. Sato*. Department of Psychology, 1 Univ. of Colorado, Boulder, CO, 80309 and Laboratory of Molecular Pharmacology(2), Bioisom Medical Research, Kobe Univ., Kobe 657, Japan.

Alterations in excitatory and inhibitory neurotransmitter activity have been shown in humans with epilepsy and in animal models of the disorder. In general, there is evidence for upregulation of excitatory transmission coupled with downregulation of inhibitory transmission. While these changes have been noted in terms of receptor function, little is known about seizures and amino acid transporters. Glutamate and GABA are each taken up by specific subtypes of transporters located on pre- or post-synaptic terminals or glia. Alterations in the distribution or concentration of these transporters in an animal model of epileptogenesis (electrical kindling) should provide clues as to how the brain responds to recurrent seizure activity. To this end, animals were chronically implanted with a bipolar stimulating electrode in the amygdala and kindled once daily until each responded with a Stage 5 seizure. One week later, animals were sacrificed and prepared for immunocytochemical examination of the cellular distribution of the glutamate and glutaetone transporter subtypes. The glial type of glutamate transporter (GLT-1) was increased in the kindled side of the piriform cortex, while no significant change was observed in other glutamatergic transporters. None of the three GABA transporters showed obvious change after kindling. These data will be discussed in the context of how alterations in amino acid transmitters might underlie the enhanced seizure susceptibility that is produced via the kindling process.


Repetitive audiogenic seizures (AGS) result in expansion of the brainstem neuronal network to forebrain structures, including the medial geniculate body (MGB). We previously observed an increase in acoustically-evoked action potentials (AP) in inferior colliculus (IC) neurons with such an AGS kindled in genetic rat epilepsy-prone rats (GPR-9). The medial part of MGB (MGM) receives input from IC. The present study examined acoustically-evoked MGM neuronal responses in kindled GPR-9 rats as compared to non-kindled rats during a period both before and after an AGS. Each stimulus rat received the same stimulus protocol (122 dB SPL) twice daily until the onset of convulsion (GPR-9 mean latency:14.5 sec) or for 15 sec in control. Each kindled GPR-9 exhibited 14 AGS. Other GPR-9s (non-kindled) were used after one AGS. MGM extracellular AP were recorded in anesthetized (ketamine/xylazine, 15mg/kg; i.p.) rats 24h after the last stimulation. Acoustic stimulation consisted of 50 stimuli (½ sec, 12kHz tone burst) effective in evoking AGS. Responses were analyzed using poststimulus time histograms. Results showed that in 11 rats in kindled GPR-9.10 neurons in non-kindled GPR-9, and 10 neurons in normals. Over 50% of MGM neurons in the GPR-9 showed burst firing, as compared to 10% of non-kindled GPR-9 and normal MGM neurons. The mean number of AP per PSTH of MGM unit bursts was significantly elevated in the kindled GPR-9 (209±47.5, S.E.M.) at 68 dB SPL in comparison to non-kindled GPR-9 (97.1±49.7) or control (71.9±19.3). The burst responses in GPR-9 may be related to changes in neuronal mechanisms of AGS kindling that subserves the increase in AGS severity (Support NIH NINDS NS 21281)
381.1 PROEPILEPTIC EFFECT OF VALPROIC ACID IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. S. Room* and K. K. Alkadhi. Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77004-5515.

This study was designed to investigate a proepileptic effect of valproic acid (VPA) in hippocampal slices. Studies were performed on brain slices from male Sprague-Dawley rats using conventional intracellular recordings in hippocampal CA1 pyramidal neurons. Action potentials were evoked in response to brief intracellular current pulses (20 ms, 0.5 nA). Veratridine (0.2-0.3 pM) was used to induce epileptiform discharges. Prolonged washing restored the rhythmic bursting. Membrane potential and membrane input resistance were measured before and after the epileptic phase of the drug in the presence of veratridine. VPA (0.5-2 mM) produced a small but non-significant decrease in membrane input resistance accompanied by a small (not significant) membrane depolarization in the veratridine-pretreated neurons. These results indicate that VPA in large concentrations has a proepileptic effect in rat hippocampal CA1 pyramidal neurons treated with veratridine.


Nowadays carbamazepine (CBZ) is established in the treatment of epilepsies as well as of affective and schizoaffective disorders. In former studies of the group calciumantagonistic effects of CBZ were found to be a possible mechanism of action in both diseases. In this study we investigated a possible interference of the action of CBZ on GABAAergic neurotransmission, since an attenuation of GABAAergic inhibition is discussed to be involved in both illnesses. Extracellular recordings were carried out from areas CA1 and CA3 of the hippocampal slice (guinea pig) GABA and its subtype agonists were applied by conventional local pressure application technique. CBZ was systemically administered. Results: 1) CBZ produced a small increase in the amplitude of field potential changes (FPC) induced by GABA (9 % after the 0.5th application during 30 minutes, n = 18). 2) CBZ showed no effect on FPC induced by the GABA-agonist muscimol (n = 5). 3) CBZ produced an increase in the amplitude of FPC induced by the GABA-agonist baclofen (18 % after the 0.6th application during 30 minutes, n = 12). The effects were completely reversible. This may be explained either by a direct action on GABA<sub>A</sub>-receptors, a positive feedback caused by presynaptic GABA<sub>A</sub>-autoreceptors or by an interference with postsynaptic intrinsic mechanisms. Further studies must reveal whether this effect contributes to the beneficial effect of CBZ in both diseases.

381.5 AUTORADIOGRAPHIC STUDY OF FELBAMATE EFFECTS ON GABA<sub>A</sub> AND NMDA RECEPTORS. A. Kume* and R. A. Prentin. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104.

Antiepileptic drug felbamate effects on GABA<sub>A</sub> and NMDA receptors were investigated autoradiographically in rat brain. For GABA<sub>A</sub> receptor, felbamate produced dose-dependent inhibition of [H]-buthylbicycloheptabenzone (BTOB) binding with IC<sub>50</sub> values approximating 5000 pM. Saturation analysis in the presence of felbamate revealed a change in Kd and B<sub,max</sub>. Dissociation initiated by picrotoxin was accelerated by felbamate. The regional pattern of [H]-BTOB binding inhibition was similar to the rat brain autoradiograms. For NMDA receptor, felbamate produced dose-dependent inhibition of [H]-MK-801 binding with the maximal inhibition of 80% at 1-3 mM and IC<sub>50</sub> values approximating 5000 pM. Saturation analysis in the presence of felbamate revealed a decrease in Kd and B<sub,max</sub>. The regional pattern of [H]-MK-801 binding inhibition by felbamate was heterogeneous. [H]-Glycine binding was not affected by felbamate. High-dose glycine only partially antagonized the felbamate inhibition but was heterogeneous. Combined our results with reported electrophysiological data suggests that felbamate effects on both GABA<sub>A</sub> and NMDA receptors at clinically relevant concentrations. Supported by NS19613, AO08671 and Tourette Association Fellowship.


The anticonvulsant drug valproic acid suppressed the NMDA induced [3H]-NE efflux in rat cortical slices (Brown et al., 1994). Chronic incubation of gloma cells with valproic acid (0.6 mM) resulted in decreased PKC activity in both mono- and biphasic cytosolic fractions (Chen et al., 1994). Incubation with 1 μM phorbol 12-myristate 13-acetate (PMA) for 10 min resulted in a loss of PKC activity in the cytosolic fraction with a concurrent increase in membrane-associated PKC activity (Watson et al., 1994). We investigated the effect of valproic acid on the PMA-induced translocation of PKC from cytosol to membrane, and the effect of valproic acid on NMDA EPSP. Brain slices (350 μm) from the hippocampus of adult Long. Evans hooded rats were stained. Control slices were incubated in oxygenated ACSF, and experimental slices were incubated in ACSF containing 100 μg/ml (0.6 mM) valproic acid for 20 min and 1 μM PMA for 10 min. After the treatment, hippocampal slices were washed in PBS, homogenized, and prepared for PKC phosphorylation assay (PKC assay kit, Gibco Co.). 100 μM hippocampal slices were used for extracellular NMDA-EPSP recordings with 100 μM bicuculline and 20 μM D-1XO in ACSF. The experimental slices were perfused with valproic acid. Results showed that PMA increased PK activity in the membrane fraction more than in the cytosolic fraction. Valproic acid decreased this enhancement by PMA, and it also suppressed the population NMDA-EPSP amplitude by 10%.


Levetiracetam is a highly effective novel antiepileptic with an unknown mechanism of action. To test the hypothesis that levetiracetam exerts its actions by an interaction with gabaergic or glutamatergic neurotransmission, intracellular recordings were obtained from the CA3 pyramidal cell layer of transversal slices of rat hippocampus. Synaptic potentials were evoked by stimulation of the septal pathway. Levetiracetam in a concentration of 10 μM did not significantly alter the membrane potential, input resistance or the amplitude of the normal subthreshold synaptic potential consisting of a fast EPSP and IPSP. In contrast, the area under the evoked epileptiform response correlated highly with the presence of the drug. This inhibition would be due to an enhancement of slow synaptic inhibitory transmission or a decrease of slow synaptic excitatory transmission by levetiracetam. Levetiracetam (10 μM) did not alter the response to 3-10 μM baclofen. Experiments on a possible interaction of levetiracetam with NMDA- or metabotropic glutamate mechanisms are currently in progress. Supported by UCB Pharma.
EPILEPSY: mechanisms of dehydrogenase MD potential compound. The effect on action potentials of sodium channels by either compound. The time course of individual currents in response to depolarizing pulse was not affected by either drug. However, the slow inactivation of sodium was prevented by the addition of steady-state slow inactivation was enhanced in the presence of either drug. In addition, both compounds caused an accumulation of sodium channel block with high frequency stimulation. Therefore, the results of the present study indicate that the effects of the two compounds on sodium current density directly reflect that ramucic acid hydrochloride and its metabolite, PPL 12495, act to block sodium channels. This may explain their ability to suppress epileptic activity.

EFFECT OF GABAPENTIN (GBP) ON AMINO ACIDS (AA) IN RAT HIPPOCAMPAL SLICES IN VITRO. (A.P. Taylor, W.J. Moncada, and H.J. Kupferberg).--Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892 and Dept. Neurosci. Therapeutics, Parke-Davis Research, Ann Arbor, MI 48105.

GBP (1-carboxyethyl)glycine/serine/lysine Acid, Neurontin®) is a recently approved anticonvulsant, but its mechanism of action remains unclear. The balance of excitatory and inhibitory inputs in the hippocampus (GABA, GLU, GABA-GLU, L-glutamate, GABA, and L-aspartate, ASP) neurotransmitter AA is important for normal neurological function and may play a crucial role in the pathogenesis and potential treatment of epilepsy. This study examined the effects of GBP on these amino acids as well as L-glutamine (GLN) which is closely related to them by the "GLU/GLN-GLU" cycle. Because of compartmentation, concentrations of GBP AA were examined in addition to the total tissue (BASAL) AA. Isotopic enrichment, after incubation with stable isotopically labeled precursors ([15C]glucose, [15C]GLU, [15C]-GABA, or [15N]-GLN) was used to measure NEU amino acids. BASAL and NEU amino acids were determined by GC/MS. The data suggest that GBP does not influence GLU decarboxylase, GLU dehydrogenase or GABA transaminase in this paradigm. However, GBP consistently decreased both BASAL and NEU GLN by about 20-30%. This effect may be due to the inhibition of the system L neutral amino acid transport. In the presence of [15C]-N-leucine, decreased decrease of the glucose cycle. Previous studies suggest that GBP inhibition of system L transport or cytosolic branched-chain amino acid aminotransferase.

EFFECTS INDUCED BY GABAPENTIN ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF BURSTING NEURONS IN THE RAT SUBICULUM IN VITRO. H. Kawasaki, D. Majewska, C. Zueko, A. Ledon, and M. Astorg. National Institute, Dept Neurology and Neurosurgery, McGill University, 3851 University St, MONTREAL, QC, Canada H3A 3B4

Sharp-wave-epileptiform activities in currents clamp mode were made from pyramidal-like bursting cells of the rat subiculum in a slice preparation to study the effects of the antiepileptic drug Gabapentin. Gabapentin (100µM) induced a small increase in firing rate (4.3±2.1 spikes s⁻¹) and in amplitude of the extracellular membrane potential (RMP in control -60±4.6 mV and reduced the ability of these neurons to generate bursts of action potentials during depolarizing current pulses (n=6). This effect was caused by a voltage-gated, Na⁺-mediated mechanism (J Neurophysiol 70 (1993) 1244-1248). The effect on bursting was also seen when the RMP was brought to control values with intracellular steady injection of depolarizing current. In 11 out of 15 cells, Gabapentin decreased by 16.1±9.7% that the sag is recorded in subicular neurons during hyperpolarizing current pulses. This sag was reduced and eventually blocked by extracellular application of Cs⁺. In this effect, Gabapentin blocks currents became larger in amplitude, the tendency to generate reboared bursts was decreased by Gabapentin. The effects of Gabapentin were still observed up to two hours after washout. Our findings indicate that Gabapentin can modify the intrinsic excitability of rat subicular neurons. Some of these effects might be caused by the blockade of an inward current that in current clamp recordings is apparent during hyperpolarizing current pulses, while the burst depression might be due to an interaction of Gabapentin with voltage-gated sodium channels. Supported by MRC of Canada.

TRAUMA: TREATMENT I


Intracisternal glutamate receptors play a significant role in secondary injury processes after spinal cord trauma. We have shown that both NBQX, a potent and highly selective antagonist of the AMPA/kainate subtypes of glutamate receptors, and CPP, as an analog of NMDA receptors, totally administered at 1 mg/kg after a standardized traumatic spinal cord injury (SCI), result in an increase in the speed of recovery and reduction in long-term hindlimb deficits. NBQX is also effective when administered after SCI has resolved [4]. The present study was performed to examine the effects of GBP on these two amino acids as well as L-glutamine (GLN) which is closely related to them by the "GLU/GLN-GLU" cycle. Because of compartmentation, concentrations of GBP AA were examined in addition to the total tissue (BASAL) AA. Isotopic enrichment, after incubation with stable isotopically labeled precursors ([15C]glucose, [15C]GLU, [15C]-GABA, or [15N]-GLN) was used to measure NEU amino acids. BASAL and NEU amino acids were determined by GC/MS. Due to the known delay in its onset of action, GBP (50 or 100 µM) was added at least 60 min before the incubation with labeled precursors. In some cases, animals were also given a 100 mg/kg i.p. bolus of GBP 1 hr prior to slices. There was no effect of GBP on the uptake of [15C]-GABA or [15C]-GLU or conversion of [15C]-GLU to [15C]-GABA. Therefore, these data suggest that GBP does not influence GLU decarboxylase, GLU dehydrogenase or GABA transaminase in this paradigm. However, GBP consistently decreased both BASAL and NEU GLN by about 20-30%. This effect may be due to the inhibition of the system L neutral amino acid transport. In the presence of [15C]-N-leucine, decreased decrease of the glucose cycle. Previous studies suggest that GBP inhibition of system L transport or cytosolic branched-chain amino acid aminotransferase.

TRAUMA: TREATMENT II

EFFECTS OF THE-23129 (ADD 230001) AND STANDARD ANTICONVULSANTS ON THE PAIRED-PAUSE PARADIGM IN RAP HIPPOCAMPUS IN VITRO. (D.W. Morgan, M. Kapatos, and H.J. Kupferberg) Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

The Antiepileptic Drug Development (ADD) Program is investigating the mechanism(s) of action of clinically used and experimental anticonvulsant drugs and methods to manipulate their effects. The paired-pause paradigm consisted of measuring the percent increase of the second CA1 EPSP and population spike (PS) after two consecutive stimulations separated by a 100 ms interval. Results were compared before and after drug addition to the ACSF in a standard submerged slice chamber. The results indicate that the standard anticonvulsant drugs, phenytoin and VPA, had minimal effects on paired-pause facilitation of both PS and EPSP. The experimental drug, D-25443, the hydrochloride salt of D-23129 (N-[2-amino-4-(4-fluorobenzyl)phenyl]carboxylic acid ethyl ester) from AWD GmbH/ASTA Medica AG, Germany, did appear to attenuate the facilitation of the conditioned PS and to a lesser extent, the EPSP slope. The attenuation was greatest at half maximum stimulation. While these data might be indicative of a D-23129 presynaptic effect, the different time profile for EPSP attenuation indicates other mechanisms may be involved. These results do show that it is possible to differentiate among anticonvulsant drugs using the paired-pause procedure, permitting speculation on possible mechanisms of action.


This study assessed the effects of theophylline on functional hemidephaptic recovery after C2 spinal cord hemisection in adult female rats using electrophysiological techniques. Twenty-four hours following anesthesia with chloral hydrate (400 mg/kg, i.p.) and C2 spinal hemisection, rats were reanesthetized. A tracheotomy and bilateral vagotomy were performed, and the femoral arteries and jugular veins were cannulated for monitoring and drug administration respectively. The phrenic nerve ipsilateral to cord injury was placed on bipolar recording electrodes. The animal was then paralysed and artificially ventilated. The ventilator was turned off and spontaneous activity recorded until ceased. After 30 min, theophylline was administered and the procedure repeated. In another group (spontaneously respiring), both hemidephaptic and diaphragmatic activities were monitored after 60 min of drug administration. In a third group of rats (spontaneously respiring), activity in both hemispheres was recorded simultaneously.

In these experiments, (n=6) theophylline induced biphasic effects, i.e. at a low dose (15 mg/kg) it evoked excitation while at a high dose (30 mg/kg) depression predominated. In the first group of spontaneously respiring animals (n=5), a standard dose of theophylline (15 mg/kg) induced activity in both hemidepethastic ipsilateral to the hemisection. In the second spontaneously respiring group (n=6), activity was induced in the nerve ipsilateral to the hemisection and enhanced in the intact phrenic nerve for up to three hours.

These results show for the first time that theophylline can activate a latent motor pathway and thus restore the respiratory drive to phrenic motor neurons which was lost by spinal cord injury. Respiratory activity is not only reinstated in the phrenic nerve made quiescent by hemisection, but it is also enhanced in the contralateral phrenic nerve. SUPPORTED BY NIH (R01NS34578) and Grant HD 31550.
TRAIINA: TREATMENT I


Past studies suggest that hypothermia, in the form of local spinal cord cooling, is beneficial in SCI. These studies report that accidental cooling to the spinal cord and temperature (10°C) was lowered in the range of 10°C. Recent studies in experimental brain ischemia have shown that modest lowering of brain temp (1-3°C) significantly lessens the degree of ischemic damage. Such decreases may be achieved by using systematic hypothermia, which in contrast to local cord cooling does not require surgical access to the spinal cord. To investigate if modest decreases in temp are also beneficial in SCI, a spinal cord injury model was used. Animals were assigned by weight, and operated on with the use of a Matruloc plate. The injury was then assessed by a spinal cord rating system. No significant differences were found between the groups, suggesting that modest hypothermia does provide some benefit in SCI.

383.4 REDUCTION OF NaCl AMELIORATES ULTRASTRUCTURAL DAMAGE TO DENDRITOMIZED MAMMALIAN SPINAL NEURONS. D. G. Emery*, J. J. Roseberg and J. T. Lucas. Dept of Zoology and Genetics, Iowa State Univ, Ames, IA 50011 and Dept of Physiology, Ohio State Univ, Columbus, OH 43210.

In low Ca++ conditions NaCl concentrations of 80 mM or greater are shown to inhibit Ca++-induced dendrotoxic damage. However, the effect of NaCl on the ultrastructural damage caused by Ca++-induced dendrotoxic damage is unknown.

The effect of NaCl concentrations on the ultrastructural damage caused by Ca++-induced dendrotoxic damage was assessed in cultures of spinal cord neurons. The cultures were treated with 100 mM NaCl for 24h followed by rewarming to 37°C and then treated with Ca++-induced dendrotoxic damage. The ultrastructural damage caused by Ca++-induced dendrotoxic damage was reduced by 50% when the cultures were treated with 100 mM NaCl. In addition, the ultrastructural damage caused by Ca++-induced dendrotoxic damage was reduced by 75% when the cultures were treated with 150 mM NaCl. These results suggest that NaCl concentrations of 80 mM or greater are effective in reducing Ca++-induced dendrotoxic damage.

386.7 COMPARISON OF SCIENTIFIC NERVE GRAPHS AND SYNTHETIC TUBES AS BRIDGES IN SPINAL CORD INJURY REPAIR. A. C. Lane* and J. M. Erb. Rehabilitation R&D Center, Edward Hines Jr. VA Hospital, Hines, IL 60414 and Department of Anatomy, Rush-Presbyterian St. Luke Medical Center, Chicago, IL 60612.

Although it has been shown that central nervous system neurons are capable of vigorous attempts at regeneration, functional regeneration is seldom successful because of the functional importance of axons. It is also known that the presence of inhibitory factors is necessary for regeneration to occur. There are few exceptions to date in experimental models. Peripheral nerve grafts have been shown to provide functional regeneration to the optic nerve and other peripheral nervous system axons. However, the supply of peripheral nerve to use as a graft is very limited. Therefore, synthetic tubular probes are being investigated as a means of enhancing and stimulating regeneration of neurons in the corticospinal tract of the spinal cord following injury. In these studies, the corticospinal tracts of rats were cut in the midlumbar region of the spinal cord. At the site of injury one end of an artificial nerve consisting of 1.2 mm i.d. polyethylene tube (Amicon) filled with 30% Marigel was implanted at the lesion site. The other end of the tube was implanted deep into the spinal cord to approximate the ventral horn at a site caudal to the initial injury. Regeneration of axons within these tubes was compared to regeneration of axons within a similar graft of fresh or prefrozen, isometric peripheral nerve. Observations were made by immunocytochemical and morphometric evaluations.

The synthetic tubes supported a greater number of cells compared to the growing axons. The nerve grafts contained many neurites which were evaluated by either Schwann cells or myelin.
383.9

Exposure of rats to a complex environment (CE) can alter brain anatomy by increasing brain weight, cortical thickness, dendritic branching and vasculature. Environmental enrichment has been implicated in the protection from lesion-induced functional impairments in rats. An animal model for cortical contusion has been developed which manifests many of the neuropathologies seen in human closed head injury. The model involves an electronic controlled pneumatic impact device which can deliver very precise and controlled cortical contusions to an animal. This model can be used to study the effects of environmental enrichment on neurological recovery following contusion injury.

Weaning CE rats were housed together in a large cage filled with novel objects that were changed daily. Isolated condition (IC) rats were kept in standard laboratory cages without the objects. Forty days following CE or IC rearing the rats were anesthetized and subjected to a unilateral cortical injury. Seven days after brain trauma, the animals were tested for spatial memory in a Morris Water Maze. The CE group was significantly better on the spatial memory task. Morphological analysis revealed a significant increase in the cortical thickness for the enriched animals as compared to those in the isolated condition.

While it is unclear at present why the CE group performs better, these animals may have a better General Adaptive Capacity (GAC). The complex environment increases the GAC and thus allows the CE animals to perform better. These animals appear to show a greater compensation ability following the trauma. Supported by NS31220

383.11
AMPHETAMINE ENHANCES METABOLIC RECOVERY FOLLOWING MODERATE AND SEVERE CONCUSSIVE BRAIN INJURIES IN ADULT RATS. A. Prikk, S.M. Lee, N. Baba, D.A. Hendry, and D.P. Becker. Division of Neurosurgery, UCLA School of Medicine, LA, CA 90024.

Catecholamine depletion, via 6-hydroxydopamine (6OHDA) administration, has been shown to alleviate the metabolic depression typically seen after brain injury in the rat. Previous work from our laboratory has shown that following a modest fluid percussion brain injury, the local cerebral metabolic rates for glucose (LCMRGlc) remain depressed as compared to sham-injured values in several neocortical regions up to 10 days post-injury. We have also found that increasing the levels of severity of F-P brain injury results in a prolonged metabolic depression. In this study, we assessed whether AMPH administration after F-P brain injury would enhance metabolic recovery from a F-P brain injury, in a dose-dependent manner, depending on the level of severity of injury. Fifteen adult male Sprague-Dawley rats were F-P injured as moderate or severe injury levels while under general anesthesia. AMPH (2 mg/kg BW) was administered 3h after injury for single treatment experiments and 3, 6, and 9 h after injury for multiple treatment studies. After 1 day post-injury, LCMRGlc was determined by 

383.12

The pro-inflammatory effects of Bradykinin (BK) B2 and B2 receptor stimulation have been extensively studied in models of peripheral inflammation. However all of the compounds available to act as BK antagonists primarily block the B2 receptor. We have investigated the development of hyperalgesia and fever following i.c.v. injections of E. Coli lipopolysaccharide (LPS). Rats received a single i.c.v. injection of LPS (200 ng) under enflurane anesthesia. Rectal temperature (RT), thermal hyperalgesia and mechanical hyperalgesia were measured before and at 2 h intervals following LPS injection (n = 8/group). RT, thermal and mechanical hyperalgesia were maximal 6 h after LPS injection. LPS-induced increases in RT, thermal and mechanical hyperalgesia were reversed (P < 0.05) by the i.c.v. co-administration of the B2 receptor antagonist, HOE 140 (10-30 pmol), but not by co-administration of B1 receptor antagonists, des-Arg9-Lys-BK (0.1-1 nmol) or des-Arg9-HOE 140 (0.1-1 nmol). Systemically administered HOE 140 (0.01-1 nmol/kg, i.p.) produced no significant effect. However LPS-induced fever, thermal and mechanical hyperalgesia were inhibited by either i.c.v. (10 nmol) or i.p. (0.1-1 nmol/kg) administration of HOE 140. These results indicate that administration of endothelin to the CNS causes the development of hyperalgesia and fever and that these responses involve the activity of kinins, via the stimulation of centrally located B2 receptors, and the formation of prostanooids.

383.13
ADENOSINE PROVIDES PROTECTION FROM CA1 TRAUMATIC NEURAL INJURY TO HIPPOCAMPAL SLICES. S.J. Sternson*, K.L. Panzir and R.A. Waling, Dept. of Neurology, UCLA, Los Angeles, CA 90024 and Sepulveda VAMC, Sepulveda, CA 91343.

Extracellular increases in adenosine concentration during hypoxia-ischemia have been found to be neuroprotective. Several cytoprotective mechanisms mediated by hypoxic-ischemic injury have been shown to be active in traumatic neuronal injury as well. Therefore, to assess the possible neuroprotective role of adenosine against CA1 neuronal injury, we investigated whether exposure to adenosine would prevent CA1 neuronal injury from fluid percussion trauma in the hippocampal slice. Treatment with 1.0 x 10-6 M adenosine began within one minute after trauma. Improved recovery at 95 min. of CA1 cortical organization (PS) from 11% ± 5 to 91% ± 4, and improved recovery of CA1 adenosine phosphate from 13% ± 2 to 91% ± 101% was observed when compared with untreated groups. This increase paralleled the production of both ATP and ADP during one hour of additional monitoring consistent with preserved long-term potentiation. Neuroprotection against trauma was also seen with the A1 receptor agonist, N6-cyclopentyladenosine (CPA) which increased CA1 P4 phosphorobic and adenosine recoveries to 93% ± 2 and 92% ± 3, respectively. These findings indicate that direct adenosine application and more specifically, A1 receptor stimulation had a greater protective effect against the development of neuronal injury after head trauma. Supported by the VA Research Services.

383.14

Ischemia and reperfusion promotes neutrophil migration into brain parenchyma, resulting in free radical formation which is in part responsible for neuronal injury. Traumatic brain injury is frequently associated with ischemia. We examined the effect of blockade of cell adhesion molecules (CAM) on the migration of neutrophils into the brain following fluid percussion injury (FPF) in rats. FPF was associated with a 25% reduction in the number of CAM-positive cells in the ipsilateral hippocampus of rats injected with antibodies to P-selectin (clone HI343). P-selectin antibody was used as controls. Thirty-two adult male Sprague-Dawley rats were randomly divided into four groups: injury-only, injured/inert vehicle, injured/CAM antibody, sham injury (all n=8). Rats were given moderate FPF with vehicle or the treated with the hi-343 inhibitor at 1 sec from saline injection. Subjects were "place" tested in the Morris water maze at 7 and 14 days post-injury for spatial learning. Rats were perfused at 14 days post-injury and their brains sectioned. Immunocytochemistry was performed on the hippocampus for ChAT and GABA, and on the hippocampus using GFAP, OX-42, OX-6, and ED-1. A separate group of 15 rats underwent injection for myeloperoxidase (MPO) determination in the brain parenchyma. Rats in the injured-only group performed poorly compared to the sham-injured group (p<.01), indicating a spatial learning deficit. Using multiple performance indices, a slight treatment effect was seen for rats in the CAM-blockade group (p<.05) in cumulative distance and quadrant time measures; however, values were not significant. No significant differences were observed in any performance index between the group antibody treated rats and the vehicle treated rats. This study suggests that administration of a CAM-blockade after fluid percussion may attenuate the behavioral effects of TBI. Supported by NIH P50 NS30505.

The therapeutic potential of various neurotrophic factors in the treatment of the injured central nervous system is widely recognized (Barinaga, et al., Science 264:772-774, 1994). The use of liposome-mediated gene transfer may limit its application in diseases caused by genetic defects. However, liposomal transfection of neurotransfactors may prove useful for treatment of CNS injury. Our laboratory recently enhanced neurotrophic effects in traumatized sensorimotor cortex by transfection of neurotrophic factor genes into the injured area. (Though the effects of liposome-mediated gene transfer may limit its application in applications caused by genetic defects, such neurotrophic agents may be useful as therapeutic agents for the treatment of CNS injuries.)

PHARMACOKINETICS following PERCUSSION INJURY. AUC, Cmax(15+10), and T1/2(77+8*) were determined 24 hours after injection. The AUC of N=350-350 (N=44); animals were treated with amphetamine, enhanced the release of neurotrophic factor(s), and improved memory performance. Amphetamine improved memory performance 24 hours after injection, compared to the saline-treated control group. In the non-traumatized control, N=12 or 10 alone, or prazosin (4 mg/kg, N=11) or methoxamine (10 mg/kg, N=10). None of the above drugs significantly affected the beam walking ability of animals during the test. (This test was designed to assess the mouse's ability to walk on a beam.) None of the above drugs significantly affected the beam walking ability of animals during the test. (This test was designed to assess the mouse's ability to walk on a beam.)

THE LIMITS OF LIPOSOMAL TRANSMISSION OF BDNF cDNA ENHANCES RECOVERY OF NEUROFOILM LOSS AND CHOLINE ACETYLTRANSFERASE (CHAT) ACTIVITY AFTER INJURY TO SEPTO-HIPPOCAMPAL CELL CULTURES. P. L. Hayes, R. J. Yang, J. S. Whiton, J. L. J. Xue, C. C. DiStefano, J. C. Clifton, A. Kingham, Dept. of Neurosurgery, University of Texas School of Health Science Center, Houston, TX 77030.

Enhancing the availability of neurotrophins following brain injury may have significant therapeutic potential. We, thus, used primary septo-hippocampal cell cultures to study liposome-mediated BDNF cDNA transfer after depolarization injury (6.0 min depolarization with 60 mM KCN in the presence of 2.8-5 mM Ca2+). BDNF or NGF cDNA was subcloned into a unique Mst site under the control of the CMV promoter (C/MV). BDNF (cDNA was completed with liposomes (1.0-3.0 μg liposome:10 μg cDNA) and transferred to septo-hippocampal cell cultures one day after neuronal injury. Transfer control cultures were incubated with liposomes without cDNA. Three days after depolarization injury, CHAT activity was determined, and Western blot and immunohistochemical analyses examined lesions of neurotrophin proteins. CHAT activity following depolarization injury. Depolarization culture transfected with BDNF cDNA did not reduce any significant CHAT activity loss. Depolarization also produced significant loss of neurotrophin proteins in non-transfected cultures. Both Western blot analyses and immunohistochemical studies confirmed that BDNF cDNA transfer significantly enhanced the recovery of neurotransmitter proteins 3 days following depolarization injury. Liposome-mediated transfected with BDNF cDNA may be used for treatment of cytokine derangements and disturbances in cholera neurotransmission following central nervous system injury (also see Yang, et al., this meeting). (Supported by NIH grants PO1 NS31998 and ROI NS21458.)
833.1 NERVE GROWTH FACTOR ATTENUATES THE LOSS OF CHOLINERGIC NEURONS IN THE MEDIAL SEPTAL NUCLEUS WHICH OCCURS AFTER FLUID-PERCUSION BRAIN INJURY IN THE RAT. [S.W. Bannister, E.S. Flamm*, T.K. McIntosh, Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

Neurotrophins have potential therapeutic applications in a number of neurodegenerative diseases. Previously we have shown that cortical infusions of NGF can attenuate memory deficits in rodents subjected to lateral fluid-percussion brain injury. This study attempts to establish a histopathological correlate for these cognitive improvements.

Male Sprague-Dawley rats underwent lateral fluid-percussion brain injury of moderate severity (2.1-2.5 atm.). Twenty-four hours after injury, a mini-osmotic pump was implanted to infuse NGF (n=9) or vehicle (n=5) directly into the region of maximal injury. Infusions continued in all animals for 2 weeks, at which time the pumps were removed. Two weeks later (4 weeks after injury) the animals were sacrificed and compared histopathologically with uninjured animals (n=5). Measurements of the area (mm2) of the septal nuclei demonstrated significant losses in all injured animals (p<0.05).

Histopathological examination of cholinergic neurons in the medial septal nucleus also demonstrated a significant loss of these neurons in all injured animals (n<0.05). The loss of these cholinergic neurons was significantly less in those injured animals which had received NGF infusion after injury (p<0.05). Acetylcholinesterase histochamical staining in the hippocampus was also greater in those animals which had received NGF infusion (but still less than uninjured animals). No differences in the size of the cortical injury were noted.

These data suggest that NGF administration, beginning 24 hours after fluid-percussion brain injury, attenuates the loss of cholinergic neurons in the medial septal nucleus. (Supported, in part, by NIH NS26818 and 08803)

833.3 COMPENSATORY PLASTICITY OF THE BRAIN AFTER TRANSCRANIAL POLARIZATION IN CHILDREN WITH CEREBRAL PALSY. M.Singh*, D.Piszczek, M.Koblikova, E. Sidikovova, Pavlov Institute of Physiology and Pharmacology. Local transcranial polarization was used for correction of motor dysfunctions in children with CP. The state of the brain and spinal cord were examined by analysis of EEG, EMG and habitus was recorded by new miometer that was developed in the lab. of Ontogenesis in the Inst. of Exp.Med. The clinical effects were depended on scalp location of electrodes, the method (mono or bipolar) of stimulation, and the intensity of the current. It was shown that the polarization of the brain has led to the decrease in the variation of EEG, restoration of stability of the interhemispheric connections, formation of normal patterns of EEG and increase in the role of T.e-rhythm. We estimated that the manipulative test, the habituation process on verbal stimulation, asymmetry of the brain changed after polarization, parallel with the restoration of motor functions. Our methods allow for the control of the rehabilitation process dynamics, and for the compensatory ability of the brain and spinal cord to be altered.

833.5 METHYLPREDNISOLONE BLOCKS MEMBRANE LIPID PEROXIDATION BUT DOES NOT SCAVENGE FREE RADICALS. DANNIA LIU AND LIPING LI, Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77555-1143

Methylnprednisolone (MP) reduces the damage to human and experimental animals following traumatic spinal cord injury. To study the mechanisms whereby MP reduces secondary injury, proglandin F2alpha (PGF2alpha, 0.2 mM in ACSF), a pro-inflammatory membrane lipid peroxidation catalyst, was infused into the spinal cord through a microdialysis fiber. OH- formation was measured by also administering salicylate (0.5 mM in ACSF) through the fiber as a trapping agent. The production of the products 2,3- and 2,5-dihydroxybenzoic acid by HPLC and electrochemical detection. Malondialdehyde (MDA), an end product of membrane lipid peroxidation, was also measured from dyeslates by HPLC and fluorometric detection. We found that OH- and MDA both increased the response to PGF2alpha administration. In the MP-treated group, 30 mg/kg and 60 mg/kg body weight of MP completely blocked OH- formation and MDA release respectively. This demonstrates that PGF2alpha inducing membrane peroxidation to release MDA and that MP blocks the pathway of PGF2alpha OH- and subsequent MDA production. Next, we generated OH- by administering Fenton reagents into the region of the injury, then infusing the MP solution. We found that MDA dramatically increased in response to OH- generation. However, MP (30 mg/kg) had no effect on MDA release. This further demonstrated that MP was acting on the injury site but not as a free radical scavenger. Supported by the Paralyzed Veterans of America Spinal Cord Research Foundation (#1025).

833.2 MCPG TREATMENT OF TRAUMATIC BRAIN INJURY IN THE RAT. Q.Z. Gong, T.M. Delashaw, R.J. Haman, and R.C. Lynch*. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Numerous studies have demonstrated the involvement of ionotropic glutamate receptors in traumatic brain injury (TBI) pathophysiology. This study examined the involvement of metabotropic glutamate receptors (mGluRs) in TBI. Of the seven cloned mGluRs, mGluR1 and mGluR5, are coupled to phospholipase C signal transduction, whereas the other five are relatively few to couple to adenylate cyclase. Pharmacological studies have demonstrated that (+)-methyl-4-carbomethoxyphenylglycine (MCPG) acts as a selective antagonist for mGluR1 and mGluR5 while the (-)-MCPG isomer is relatively inactive.

In this study, a single 5 mg injection of (+)-mCPG (n=8, 0.2 mM, n=10), (-)-mCPG (0.2 mM, n=10) or CSF-vehicle (n=11) was administered into left lateral ventricle 5 minutes prior to TBI. A 2.1 atmosphere fluid perfusion pulse was delivered through the right lateral ventricle. Memory was indexed by placing rats in a Morris water maze. MCPG treated rats demonstrated significant impairments in acquisition and retention phases of the probe trial. MCPG treated rats had significantly smaller deficits compared to CSF-treated rats on beam-walking (P<0.05) and memory (P<0.05), but did not differ from CSF-treated rats on other systemic arterial blood pressure or heart rate responses to injury. Neither the low-dose (+)-MCPG nor (-)-MCPG affected behavioral deficits.

These results suggest that TBI-induced activation of mGluR1, and/or mGluR5, contributes to TBI morbidity. Blockade of these receptors with (+)-MCPG may reduce certain components of TBI morbidity.

Supported by NS12587 and NS29995 from NIH.

833.4 INHIBITORY EFFECT OF NYA ON NOS INDUCTION IN AN ANIMAL MODEL OF CEREBRAL HEMORRHAGIC HYPERTENSION. Z.C. Peng1, X.D. Liang2, C.C. Liu3, Z.Q. Zhang1, Z.D. Yang4, and X.Y. Hu1. 1Dept. Anesth. & Trad. Chinese & Western Med., Human Med. Univ., P.R. China; 2Dept. Anesth. & Histol., Univ. Verona, Italy; 3Acad. N.Y. (NYA), a component of the neuropeptide Y, has been found to be effective in the recovery of movement deficits in patients suffering from cerebral hemorrhage. We studied the modification of nitric oxide synthase (NOS) activity in an animal model of cerebral hemorrhage, and the effect of NYA, by means of NADPH-diaphorase (NOS) histochemistry, NYA (4.38 g/kg body weight) was orally administered to adult Wistar rats 2 h before autologous blood injection into the right lateral ventricle. NYA Group was then repeated daily until perfusion. NYA was not administered in other animals which received blood injection in the IC (C group). Rats were perfused 2, 4, 7, 14, and 28 days after operation and brain sections for NOS-Immunostaining (NOS-Immunohistochemistry), NPY-positive non-pyramidal neurons were seen in the cerebral cortex, as normally present. In addition, in the C group of rats, numerous NPY-positive pyramidal neurons and ependymal cells were seen throughout the ipsilateral and contralateral hemisphere, especially in the operated hemispheres, 2 days after surgery. NPY-positive non-neuronal cells, presumably macrophages and astrocytes, were also seen in the C group in a small number. NPY-positive pyramidal neurons were only seen in the ipsilateral cortex 4 days after the operation, when very few NPY-positive non-neuronal cells were still detectable. NPY-positive non-neuronal cells disappeared progressively 7 days after the operation and they were not visible after 28 days. Non-neuronal NPY-positive cells were not detected in the C group, in which very few NPY-positive pyramidal neurons were detected only in the area close to the needle track in the earliest stage. Thus, our results indicate that autologous blood injection into the brain may induce NOS expression, and that NYA may inhibit such induction.


We previously reported that progesterone (P) reduces cerebral edema associated with cortical contusion in male and female rats1. In males this leads to an amelioration of the amount of cognitive deficit related to neuronal death in the thalamus2. To determine whether females' injury induced cognitive deficits are reduced in the same manner as in males, Sprague-Dawley rats were given medial frontal cortical contusions on the day of proestrus. Both P or oil injections (4 mg/kg) were given beginning at 18h after injury, and each 24h for 7 days. Beginning 7 days after surgery, each rat was tested for 10 days in the Morris water maze. Contused female rats took significantly longer to use a longer path to find the hidden platform compared to sham female. Contused females receiving P injections performed better than females treated with the oil vehicle.

These data were compared to that of male rats previously report3. Sham males and females performed equally well on the MWM, however, 2 weeks after cortical contusion, oil-treated females performed significantly worse than oil-treated males. P treatment reduced this gender difference, and females performed as well as treated with P as in males. As with the males, cell density measurements demonstrated significant neuronal loss associated with the contusion in the medial dorsal thalamus (MD) as well as in cortical areas adjacent to the contusion area. Reduction of the MD cell loss was seen in P-treated lesion rats. There were no gender differences in cell loss or rescue.


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834.1 
EFFECT OF IMMUNE PRIMING ON BORNA DISEASE A. J. Lewis1, J. L. Whitton2, U. Ngo3, S. van den Noort3 and W. I. Lipkin4 Deps of Anatomy and Neurobiology1 and Neurology3, Univ. of California, Irvine, CA 92717; Scripps Research Institute5, La Jolla, CA 92037. Borna disease was first described as an encephalitic disease of horses and cattle in Western Germany. The viral agent has since been found to be a non-segmented, negative strand RNA virus. Borna disease virus (BDV) causes an immune-mediated neurological disorder in a wide range of host species. The N protein of BDV, p40, is one of the most abundant viral proteins present in infected cells and tissues and elicits a strong antibody response. A vaccinia virus vector expressing p40 (Vp40) was used to prime an immune response in male Lewis rats prior to challenge with BDV infection. Control animals received either a vaccinia vector expressing an irrelevant antigen or PBS. Two weeks later animals were challenged intranasally with BDV. Clinical observations were made daily. Animals receiving Vp40 showed intensification of clinical disease when compared with both control groups. At 14, 21, 31 and 36 days post BDV challenge brains were collected for histology and RNA analysis. Examination of inflammation in hematoyxlin and eosin stained brain sections showed increased perivascular infiltration. Northern hybridization showed a dramatic decrease in expression of viral RNAs in the experimental group. Immunization with Vp40 resulted in an apparent increase in viral clearance, however encephalitis and clinical symptoms were exacerbated.

834.3 
RETROVIRUS-INDUCED ASTROCYTIC NITRIC OXIDE (NO) PRODUCTION FOLLOWING INFECTION WITH NEUROPATHOGENIC ts1 MoMuLV: A MECHANISM FOR NEUROAL DEMENTIA AND CHANGES IN SPONTANEOUS POLIO-ENCEPHALO-MYELOPATHY OF FVB/N MICE J. M. Vann, P. Skrzek and B.R. Brooks1 Neurol & Research Svc, Wm S Middleton Memorial VAMC, Madison, WI 53705-2286 and Neurology Div, University of Wisconsin Medical School. Background: NO-mediated neuronal or oligodendroglial damage by HIV-1 in AIDS has been postulated to require NO-producing neurons or microglia from a microglial and/or astrocytic origin on astrocyte (As) inducible nitric oxide synthase (iNOS) is unknown. Methods: Subcortical As NO production in vitro was monitored by measurement of nitrite formation with Griess reagent before and after ts1 infection. Lipopolysaccharide (LPS) and gamma-interferon (gIFN) effects on mock-infected and ts1-infected As NO production were studied separately and together. Results: Basal NO production by ts1-infected As was higher than in mock-infected As from 72 hr pl. LPS (10-10,000 ng/ml), but not gIFN, increased NO production in mock-infected and ts1-infected As. LPS (1 ug/ml) and gIFN (100 U/ml) together stimulated significantly higher NO production in ts1-infected As than mock infected As. L-N-nomonomethyl-arginine (LNMMA), an inhibitor of iNOS, blocked these effects. Conclusions: NO production by retrovirus-infected As provides an added source for indirect neuronal/oligodendroglial death.

834.4 
IN VITRO AND IN VIVO ANTIVIRAL ACTIVITIES OF HUMAN RECOMBINANT MONOCLONAL ANTIBODIES TO HSV-1 AND -2 J.M. Sama1, J. A. Williams2, M. D. Long1, E.L. Bloom1 and D.R. Bangro3. Deps. of Neuropathology1, Immunology2 and Molecular Biology3. The Scrips Res. Inst., La Jolla, CA 92037. In HIV, herpes simplex virus (HSV) is an important cause of morbidity and mortality in man. Although the availability of drugs with high therapeutic indices has greatly improved the management of herpetic infections, the emergence of antiviral resistant strains, especially in the immunocompromised patients, has become a cause of serious concern and underscores the importance of developing new and alternative prophylactic and therapeutic drugs. We have isolated large panels of human recombinant monoclonal antibodies to HSV by antigen selection (phage display) from combinatorial antibody and display libraries. Some such antibodies displayed neutralizing activity in vitro. A type common antibody specific for glycoprotein D, antibody ACHS11, neutralized very effectively both laboratory strains and clinical isolates of HSV-1 and -2 and was capable of inhibiting cell-to-cell transmission of these viruses in vitro as an Fab fragment. This antibody acts through a molecular mechanism and is found monovalently at its epitope. In addition, this antibody more that doubled survival times (p<0.001) when administered to acyclogic mice infected with HSV-1. Such prologation of survival was seen even when the antibody was administrated up to 24 hours post-infection, a time when the virus is already in the CNS. To improve the implementation of this approach, we have now modified this affinity-based cloning technique to allow for more facile isolation of recombinant antibodies to specific viral proteins in their native conformation. With the modified method, phage display libraries are paired against viral glycoproteins captured from infected cell extracts with immobilized monoclonal antibodies. Using this strategy we isolated six novel neutralizing human recombinant antibodies to HSV glycoprotein D and B. Partially supported by MII 76880.

834.5 
HERPES SIMPLEX VIRUS IN POSTMORTEM MULTIPLE SCLEROSIS BRAIN TISSUE: V. J. Sanders1, A. D. Wadding2, E. L. Felsen, W. W. Tourtelotte, M.D., Ph. Neurology Research, West LA, V.A. Med. Ctr., Los Angeles, CA 90073 Objective: To test for the presence of herpes simplex virus 1 and 2 (HSV) in postmortem brain samples from patients with multiple sclerosis (MS) and controls using polymerase chain reaction/Southern blot hybridization methodology (PCR/SB). Background: 1. HSV is known to permeate CNS tissue in the presence of long latencies. It can cause focal demyelination in animals. Methods: Dissected plate tissue classified as active or inactive and unaffected white matter (WM) and gray matter (GM) from 37 MS cases were screened for HSV by PCR/SB. WM and GM from 22 Alzheimer’s disease, 17 Parkinson’s disease and 22 cases without neuro-psychiatric disease served as controls. Results: PCR screening of the MS cases and 28% (17/61) of the control cases had samples positive for HSV (p=0.10); 41% (9/22) of active MS plaques were positive for HSV. 20% (9/45) had virus; 24% (9/37) and 14% (5/37) of MS cases had HSV in WM and GM, respectively. 72% (43/61) of non-MS cases had HSV in WM and GM, respectively. These differences were found between all subgroups (p=0.07). Conclusions: HSV was present in a greater frequency of MS cases compared to control cases. A greater frequency of active plaques for active MS cases had HSV in WM and GM. This is in agreement with previous studies performed in MS WM and GM as well as controls makes an association to the MS disease process uncertain. Future: Cellular localization of virus and its relationship to pathology and latency may reveal an association with disease.

834.6 
THE USE OF A SMALL ANIMAL MODEL FOR CMV RETINAL INFECTION: IN SITU MOLECULAR BIOLOGY: W.E. Luray1, L. Epstein1, W. Ren2, B. Blumberg1, A. Jones1, M. del Campo2, J.L. Hitchcock3. 1.U. of Rochester Medical School, Rochester, NY; U. of Alabama, Birmingham School of Medicine2, Birmingham, AL. Human cytomegalovirus (HCMV) is an important cause of morbidity and mortality in patients infected with HIV, and little is known about the pathogenesis associated with this virus. No models have been developed to study HCMV infection of human neural tissue in vivo. We report on the development of an animal model which will aid in delineating this problem. We sought to detect the presence of HCMV in human retinal neovascularization in SCID mice using immunoperoxidase/ICC and in situ hybridization (ISH). As HCMV is specific for the human genome, in vivo infection of human cells into an animal model is a valuable tool in following the course of the disease. Human fetal retina was explanted into the anterior chamber (AC) of SCID mice and infected with HCMV. ICC was performed with monoclonal antibodies targeted against early, middle, and late replication phase proteins and other gene products. Peroxidase/anti-peroxidase ICC was performed using Elite Vector ABC and DAB Peroxidase Substrate Kit. ISH was performed using a 500bp random-primed digoxigenin labeled digDNA probe which targeted a specific region of CMV DNA. This technique and the process of in situ polymerase chain reaction was tested on AD 169, CoV, and Crump strains of HCMV. After days post-infection, the grapes are differentiated with detection of intracytoplasmic and extracytoplasmic viral components. ISH was successful at detecting positive cells with the use of the labeled probe which specifically targeted a region of the CMV genome. This shows that active infection is occurring in the transplanted tissue and that we are infecting human tissue with HCMV. The AD 169 strain was much more reactive than the CoV and Crump strains of HCMV. This animal model allows the study of HCMV replication in nervous tissue and the specific effects of HCMV on human retinal cells. In vitro molecular immunohistochemical and in situ hybridization techniques are suited to the detection of HCMV infected retinal cells. Here, we use the AC immune privilege and the immune status of the SCID to mimic HCMV infection in AIDS patients. Supported by NIH and Strong Children’s Research Center.
834.7

CELL-TYPE SPECIFICITY AND INDUCIBILITY OF THE HUMAN CYTOMEGALOVIRUS MAJOR IMMEDIATE EARLY PROMOTER IN BRAIN-DERIVED TRANSFERRIN+ MICE. I. M. Hayman1, S. B. Girshovich1, A. Azagi2, B. Lüscher2, and P. J. Mitchell1. 1Institute of Pharmacology, University of Basel, Switzerland; 2Institute of Molecular Biology II, University of Zurich, CH-8057 Zurich; 3Institute of Neuropharmacology, University Hospital, CH-8091 Zurich, Switzerland.

Human cytomegalovirus (HCMV) has been associated with a variety of pathologies including CNS infections. We showed previously that the HCMV immediate early (IE) promoter directs lacZ gene expression in transgenic mouse embryos specifically to sites of known targets of congenital HCMV infection in human fetuses (Koedood et al., 1995). Thus, it is possible that a specific transcription regulatory role of IE lacZ expression in choroid plexus, some endothelial and ependymal cells, and in several neuron populations, notably in brainstem, cerebellum, cerebral cortex, hippocampus, olfactory bulb, and thalamus. Double-labeling with GFAP antibody showed that the IE promoter was inactive in most astrocytes, but was dramatically induced in astrocytes of the ipsilateral cortex and striatum during gliosis induced by a stab lesion of the neocortex. The IE promoter activity pattern in the murine CNS is consistent with clinical data on human HCMV infection and supports a model for HCMV propagation in the CNS whereby astrocytes become permissive for infection in response to tissue damage caused by infection of primary targets.

834.9

EXPRESSION OF CYTOKINES AND NITRIC OXIDE SYNTHASE BY ASTROCYTES IN CHRONIC DEMYELINATION CAUSED BY MHV-JHM IN SUSCEPTIBLE AND RESISTANT STRAINS OF MICE. D. P. Fortin1, S. M. C. Tsuchida2, S. H. Huang1, and C. J. Miller1. 1Department of Pediatrics and Neurology, University of Iowa, Iowa City, IA 52242.

Intranasal inoculation of C57BL/6 mice with mouse hepatitis virus (MHV), strain JHM under the appropriate conditions results in a chronic demyelinating disease characterized clinically by hindlimb paralysis (HLP). Expression of cytokines, nitric oxide synthase (iNOS), MHC class I and II antigens and possible cellular markers were investigated in the two strains of mice. Cytokines TNF-α, IL-1β, IL-10 and iNOS were all up-regulated in the spinal cords of HLP mice as revealed by immunocytochemistry. Double-label studies demonstrated that cells expressing these cytokines and iNOS were astrocytes. Most astrocytes expressing cytokines and iNOS were not infected by virus but were distributed in the white matter of spinal cord near virus and demyelinating lesions. Also in additional experiments, the spatial relationship of virus-infected cells and the activated astrocytes was analyzed. The analysis showed that 15% of all infected cells in these chronically infected mice were astrocytes. The expression of cytokines and iNOS in astrocytes in these strains of mice is similar but not identical to findings in mice with acute encephalomyelitis caused by MHV-JHM. In acutely infected animals, TNF-α, IL-1β, IL-10 and iNOS are found mainly in macrophage-like cells. In addition, MHC class I and II antigen are also up-regulated in the spinal cords of chronically infected mice, although not in astrocytes. The results of this study demonstrate that expression of different cytokines and nitric oxide synthase in the astrocytes is a feature unique to the chronic as opposed to acute infection caused by MHV-JHM. The concordance of the present results with findings in other animal models of demyelinating disease and in patients with multiple sclerosis suggests that astrocytes play a major role in chronic demyelination.

834.11

NORMAL NEURONAL EXCITABILITY AND SYNAPTIC TRANSMISSION IN PRION PROTEIN GENE ABALTED MICE. P. M. Lloyd1, G. L. Schaub1, J. D. Unsworth1, S. B. Prat1, and R. A. Nicoll1. Dept. of Cellular & Molecular Pharmacology, Pathology & Neurology, Biophysics & Biophysics; UCSF, San Francisco, CA 94143.

The physiological function of the cellular prion protein (PrP) remains uncertain. To investigate whether the loss of PrP could affect neuronal excitability and/or synaptic transmission in the CNS, we have used hippocampal slices and recorded in vitro in the CA1 region from PrP knockout mice (PrP−/−). Field potential recordings have revealed a normal level of synaptic inhibition since in response to stimulation in stratum radiatum, responses from PrP−/− mice consisted of a single population spike similar to the one recorded in mice and the plot of field EPSP slope versus the population spike amplitude showed no difference between the two sets of data (n = 12). Intracellular recordings also failed to detect any difference in electrical membrane properties and the reversal potential for IPSPs was unchanged (-70.1 ± 1.9 mV in 10 control and -69.5 ± 2.3 mV in 8 PrP−/− mice). The study of the LTP kinetics also reveals no alteration (n = 14). In another set of experiments, we examined whether LTP was altered and found that no statistical difference existed between the two groups of animals (0.9 ± 0.2% for the control PI and 1.0 ± 0.2% for the PrP−/− mice). The level of inhibition was reported to be impaired in the PrP−/− mice (Collinge et al., Nature 332:295-297, 1994), but with the earlier detailed below impairment in which no significant result could be demonstrated (Büeler et al., Nature 356:577-582, 1992). Supported by NATO, the CNRS and the NIMH.

834.8

EFFICIENT TRANSDUCTION OF HUMAN NEURONS WITH AN ADENO-ASSOCIATED VIRUS VECTOR. B. Du, P. Wu1, D. M. Boldt-Haefeli, J. Witten, and P. J. Trojanowski. Division of Infectious Disease and Hematology/Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02115

An adeno-associated virus (AAV) vector containing a lacZ gene under the control of the CMV immediate early promoter was evaluated with respect to its transduction efficiency and integration ability in nondividing human NT neurons. Using dual staining for immunocytochemistry with monoclonal antibodies to neurofilament proteins and B-galactosidase (B-gal), we were able to demonstrate: 1) co-localization of neurofilaments and B-galactosidase in the same cell; 2) a tissue-dependent pattern of AAV vector transduction efficiency, with up to 100% of the neurons expressing B-gal. The efficiency found in NT neurons was equal to or greater than the AAV transduction efficiency in 293 cells, which are a highly sensitive cell line to AAV. This indicates that the human NT neurons are readily susceptible to AAV-mediated transduction. No neurotoxicity was detectable by a MT4 assay. Further, quantitative PCR analyses of high molecular weight cellular DNA from the transduced neurons indicated that the copy number of the AAV B-gal genome increased not only in a dose-dependent but in a time-dependent manner, the latter suggesting a slow progressive rate of integration of the vector over a period of days following transduction.

In summary, this study demonstrates that AAV-based vectors can efficiently transduce, express and integrate a foreign gene into postmitotic human NT neurons.

834.10

INTRACEREBROVENTRICULAR ADMINISTRATION OF IL-1β IN DOGS PRODUCES CSF LEUKOCYTOSIS BUT NOT FEVER. G. S. Link1, B. Goodman1, and D. P. Harley Division of Neuroradiology Critical Care, Dept. of Neurology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21287.

IL-1β has a well-recognized role in initiating and perpetuating inflammatory cellular responses and in producing fever. Clinically, fever is often the first objective sign of systemic illness. In this study we examined the effect of intracerebroventricular (i.c.v) administration of IL-1β on cerebrospinal fluid (CSF) white cell count and fever. Beagle dogs (n=6) were each surgically prepared with an indwelling i.c.v catheter system. After at least one month of recovery, human IL-1β was given i.c.v. in doses of 50, 100 and 200 ng/kg. Controls received 0.1% BSA in normal saline. Blood and CSF specimens were obtained before and for 3 hours after dosing. Body temperature was measured continuously throughout. Biofluid analyses used standard manual methods and an automated hematocrit. Blood samples were obtained from the carotid artery.

Results demonstrated a rapid increase in CSF leukocyte count. By 5 min after administration, peak cell counts were observed. For the 100 and 200 ng/kg groups, the WBCs were 36 ± 8.7 mm3 (Mean ± SEM) and 89 ± 39 mm3, respectively. These results were significantly different from control (p<0.05, t-test). Over the next hour, these levels gradually decreased back to baseline. At 5 min, the white cells were noted to be predominantly mononuclear, approx. 75% of total WBCs. Body temperature did not significantly increase from baseline.

In conclusion, following i.c.v administration in dogs, IL-1β leads to CSF leukocytosis that is predominantly mononuclear. This effect is dose-dependent. In addition, at the doses tested, there was no pyrogenic response. Thus, findings suggest that disorders that lead to a predominantly CSF mononuclear (such as septic meningitis) may be mediated by IL-1β. In addition, it appears that fever requires a higher level of this cytokine or possibly another mechanism is involved.

834.12

THE PRION PROTEIN BINDS TO PSF, AN ESSENTIAL SPlice FACTOR. Bruno Oesch1, Paul Jen1, and Edith Gubler. Brain Research Institute, University of Zurich, Auguste-Favre-Str. 1, 8029 Zürich, Switzerland, and Dept. of Biochemistry, Biozentrum, 4056 Basel

Interaction of the prion protein (PrP) with other cellular proteins on ligand blots has led to the identification of two PrP ligands of 45 and 110 kDa (P1 45 and P1 110) that are reported to represent the prion protein (PrP) as a splicing factor associated with the polyoma virus-like binding protein. P1 110 was purified using sucrose gradient centrifugation followed by chromatography on carboxyl methyl cellulose, reverse phase, and hydrophilic interaction columns. Triptoyl peptides of bovine P1 110 were sequenced revealing extensive homology to human PSF. Binding to PrP was confirmed with recombinant PSF. Using gel shift assays, we were able to show the presence of PSF in our most purified preparations of P1 110. In addition, the binding pattern of PrP to unfractioned sections was similar to the distribution of PSF i.e. the signal was stronger in nuclei than cytoplasm.

These results suggest that either PrP may have an alternative function at the membrane or that PrP is not exclusively located at the cell surface. Recently, an intracellular 60 kDa PrP isoform has been identified and known to be more abundant in scrapie-infected animals suggesting that this isoform may aid the generation of disease-specific PrP or infectious particles. We are currently investigating whether the 60 kDa form of PrP copurifies with PSF.

Previous studies have shown that 139R infection of hamsters causes obesity and marked histopathological changes in the islets of Langerhans and pituitary. Using routine EM, we now report more details of the histopathological changes in the pars distalis of the pituitaries of 139R-infected hamsters. Dilution and vacuolation were first observed in the mitochondria and rough endoplasmic reticulum (RER). The subsequent abnormal events seen included vacuolation and breakdown of the secretory vesicles, lysosomal breakdown and finally cell digestion and lysis. In some damaged cells the nucleus remained attached to the cellular membranes, suggesting that it was protected to some degree from the effects of cytotoxic polynucleotides by the nuclear membrane. Based upon these observations, we conclude that the cellular death seen in the pituitaries of 139R-infected hamsters is due to necrosis rather than apoptosis.


The present experiments investigated the novel finding that bacterial endotoxins can induce neurological symptoms in adult wistar rats. Neurological symptoms typically appeared 6-24 h post endotoxin administration (iv and ip), persisted for 24-36 h, and then spontaneously resolved. These symptoms appeared in approximately 5% of animals, although symptom severity was variable. Mildly affected rats presented with mainly proprioceptive deficits, which manifested as an abnormal gait; moderately affected rats presented with proprioceptive and mild motor deficits, and severely affected rats presented with proprioceptive, motor (paralysis/ataxia), and sensory (reflex) deficits. Further experiments were conducted to investigate the generalisation of this syndrome across a number of wistar strains, and to localise the site(s) of pathology mediating this syndrome; and, determine which endotoxin-induced chemical pathway(s) might be mediating this syndrome.

THE MOLECULAR PATHOGENESIS OF THE TUBULO-FILAMENTOUS PARTICLES: EVIDENCE FOR A HOMOLOGOUS SINGLE-STRANDED DNA IN SPONGIFORM ENCEPHALOPATHY. H. Narang*, Dept of Psychiatry, School of Medicine, SUNY, Stony Brook NY 11794.

Scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) with six other diseases have been grouped together as spongiform encephalopathies (SE). The gene susceptibility factor for SEs has been identified in human and animals. Existence of at least 20 genetically stable strains in SEs appears to be incompatible both with PPV or itself with a specific point mutation seen in the PrP gene being the agent. The evidence presented so far suggests that SE is an infectious disease and not a genetic disorder. Unique virus-like tubulofilamentous particles termed nemaviruses (NVP) have been consistently observed in SE brains by EM. In a blind study, a scrapie-infected and a normal hamster brain were examined at 3, 5, 7, 10, 14, 18, 21, 24, and 28 days post-inoculation. Examination of grids prepared from both left and right sides of the brain at 3, 5 and 7 days revealed no NVP or SAH. However, at 10 days scrapie-infected hamster brains from the inoculated right side revealed both NVP and SAH. From 18 days post-inoculation the NVP and SAH were observed in both sides of the brain which would suggest that replication of the agent at the local site of inoculation. No NVP or SAH were seen in any of the preparations from normal brains. Nucleic acid was purified from the content of enriched preparations of mitochondria/NVP. The ssDNA was synthesized into double stranded RNA and inserted in Pex18, cloned and sequenced. An unusual palindromic six base (TACGTA) 10 repeat sequence was obtained, as observed in scrapie, which revealed no significant homology to other sequences. A probe prepared reacted with a band of about 1.2 kb in scrapie, CJD and BSE but not with normal DNA specimens.


Headache and nausea are the most relevant clinical symptoms in early bacterial meningitis (BM). The hypothesis that headache in BM may result from trigeminal activation is strongly supported by observations in the early phase of experimental meningitis which is characterized by a dramatic regional cerebral blood flow (rCBF) increase and inflammation within the first 6 h. We undertook this study to examine a possible role of trigeminal afferents in the rCBF response.

In 5 male wistar rats the nasoalveolar nerve (NCN), which innervates the meninges over the frontal lobe was cut on the right side 10 days before the experiment. Meningitis was induced by intracranial injection of pneumococcal cell wall components (equivalent of 10^6 cfu Streptococcus pneumoniae PsV-527, Jena). Physiological parameters were monitored throughout the experiment; rCBF was measured continuously over 6 hours with laser Doppler flowmetry with two probes bilaterally over the right and the left frontal lobe measured through the thinned skull.

NCP cut 100 102 103 104 105

**p<.05 Students t-test, mean ± SD

Denotes increases the rCBF increase in BM. The study provides first evidence for a role of a neurogenic mechanism in rCBF response in acute inflammation of the CNS. We speculate that neuropeptides released from trigeminal C-fibers may be involved in mediating rCBF increase in BM.

PEUOCOCCAL CELL WALL COMPONENTS RELEASE TNF-a FROM CEREBRAL ASTROGLIAL AND ENDOTHELIAL CELL CULTURES. M. Weich*, D.Freyer, J.R.Webber, P.Schoel, A.Masell, K.Augustin, W.Burgt, U.Dimazl, Dept of Neurology, Dept of Microbiology, Charit?, Humboldt University Berlin, Berlin, Germany. Astroglial cells and cerebral endothelial cells have been shown to produce tumor necrosis factor-alpha (TNF-a) upon stimulation with cytokines like IFN-g, IL-1 or bacterial lipopolysaccharides (LPS). The cell wall of Streptococcus pneumoniae (PCW) has been shown to cause inflammatory reaction, which might be mediated in part by pro-inflammatory cytokines like TNF-a. TNF-a has also been found in cerebrospinal fluid in experimental meningitis and in humans.

We tested whether astroglial or endothelial cells could account for this TNF-a release. Addition of PCW to cultured rat astroglial cells increased TNF-a in the supernatant significantly after 48 h from 3 ± 0.3 pg/ml to 9.8 ± 1.6 pg/ml and was inhibited by dexamethasone (10^{-6} M). TNF-a release from astroglial cells, as determined by the L929 bioassay, was dose-dependent in a range shown to cause meningeval inflammation in vivo. TNF-a production in cerebral endothelial cells after 12 h was higher than in astrocytes (117 ± 45 pg/ml) and also inhibitable by dexamethasone.

Our results suggest astroglial, microglial and endothelial cells as sources of TNF-a, which could cause inflammatory and anti-inflammatory response in the early phase of experimental pneumococcal meningitis leading to recruitment of blood leukocytes and finally breakdown of the blood-brain-barrier (This work was supported by the DFG).

INTRACISTERNAL INJECTION OF TUMOR NECROSIS FACTOR ALPHa (TNF-a) INCREASES CEREBRAL BLOOD FLOW (rCBF). K.Agusten, M.Weich*, D.Freyer*, K.M.Binhel, P.Schoel, G.Ramstaller*, U.Dimazl, Dept of Neurology, Humboldt University, Schering AG, Berlin, Germany, Dept of Neurology, Univ. of Innsbruck, Austria.

TNF-a is a pleiotropic cytokine, which is thought to be a central mediator of various CNS diseases. Its role in bacterial meningitis, for example, is still under controversial discussion. We compared the effect of intracranial injection of recombinant rat TNF-a, rat monoclonal antibody (rMoAB) against rat TNF-a, and rat brain water content in rats. TNF-a (activity: 1300 units/ml) was applied in three doses (35 pg (n=3); 70 pg (n=4); 280 pg (n=3) per rat). In control animals saline i.c. was injected. rCBF measurement was performed by laser Doppler flowmetry through the thinned right parietal bone. Within the observed 8h post i.c. injection no differences were seen in saline treated animals to be compared to untreated rats. In TNF-a i.c. injected animals a systemic effect was seen.

Other than the 35 pg TNF-a group did not differ from saline i.c. injected. An injection of 50 or 280 pg TNF-a increased rCBF significantly beginning 2h after i.c. injection compared to the lower doses. 4.5h and later rCBF of 280 pg TNF-a group was higher than of all other groups (mean±SD 6h after i.c. injection: 0ug: 115±17; 35ug: 120±10; 70ug: 170±39; 280ug: 248±42; p<0.05. ANOVA, Student-Newman-Keuls). Animals treated with 35 pg TNF-a, 2 TNF-a, a possible mediator for systemic effects of CNS inflammation. The role of NO in TNF-a mediated rCBF response has to be elucidated in future.
834.19

TROPHISM OF TOXOPLASMA FOR ASTROCYTES IN A HUMAN FETAL NEURAL CULTURE. S.K. Halonen and P.C. Chu*. Dept. of Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10412.

Toxoplasma gondii is an intracellular parasite that is one of the most common opportunistic infections of the CNS in AIDS patients, causing an often fatal encephalitis in neuronal cells. Treatment of this disease is hampered by a lack of information about the basic biology of this parasite in neuronal cells. In this study primary cultures from human fetal brain were used to study the behavior of Toxoplasma in neuronal cells. Toxoplasma was able to infect both astrocytes and neurons but growth of the parasite in astrocytes was approximately 15-fold higher than in neurons. The behavior of Toxoplasma also differed dramatically in neurons versus astrocytes. In neurons, Toxoplasma replicated within 4-6 hrs, with an average of 22-24 parasites/host cell at 48 hrs. In neurons, however, parasites replicated only 1-2 times over this same 48 hr period. These data suggest that some of the infected neurons were necrotic 24 hrs after infection. However, approximately 10% of the infected neurons were able to support replication of the parasite. These results indicate that 1) astrocytes support the majority of the proliferation of the parasite in the brain and 2) neurons exhibit a differential susceptibility to infection with Toxoplasma. A better understanding of the tropism for astrocytes and of the differential behavior of Toxoplasma in neurons may yield insights into the mechanisms underlying toxoplasma encephalitis. (S.K. Halonen is a Aaron Diamond Foundation Fellow and this work was supported by a grant from The Aaron Diamond Foundation).

MENTAL ILLNESS—SCHIZOPHRENIA III

835.1


Growing evidence suggests that aberrant glutamatergic systems interacting with dopaminergic systems may be involved in the pathophysiology of schizophrenia. The NMDA receptor is a homo-heterotetrameric complex that has distinct domains, including NMDA and glycine recognition sites and channel binding sites. Furthermore, the subunit composition of the receptor displays regional variations in brain. [H-3]CGP39653, [H-3]dizocilpirdine and [H-3]dipiridamole were used to assay specific binding in NMDA and glycine recognition sites and channel sites, respectively, in membranes from the orbital gyrus (OG) and superior temporal gyrus of postmortem brain samples from normal control subjects and patients with schizophrenia and bipolar disease. The specific binding of [H-3]CGP39653 to OG membranes was higher in samples from schizophrenic and bipolar patients (+6%) than that in controls. The difference between the schizophrenic and control samples was significant (p<0.05 by ANOVA and Scheffe's test). There were no other statistically significant differences. Correlations between specific binding of any of the radioisopes in either brain region and the intervals between death time and freezing time were not statistically significant. These findings are consistent with frontal cortical glutamatergic hypofunction and consequent up-regulation of NMDA recognition sites in schizophrenia, but do not exclude the possibility that the effect seen is due to neuroleptic treatment.

835.3


Ten schizophrenic patients underwent prolonged [123I]D2 /SPECT studies while free from antipsychotic drugs (mean 150; range: 7-730 days) and a measure proportional to peak D2 receptor binding potential (BP) was determined as previously described (Knable, et al., J. Nc. Med., 1993). Eighteen healthy controls were studied for comparison. For schizophrenic patients, the peak BP for the left and right striata were 0.67±0.25 and 0.68±0.20, respectively, and for normal controls these values were 0.71±0.21 and 0.71±0.21. Although peak BP tended to be less in schizophrenics, this difference was not statistically significant. There were no significant differences between the left and right striata for either group. There were no significant correlations between peak BP and positive symptoms, negative symptoms, movement disorder, age, or illness duration. Four of the schizophrenic patients were able to complete two 120 /SPECT studies during the drug free period. Percent change in peak BP was significantly correlated with change in positive symptoms (rho=0.80) and change in negative symptoms (rho=0.90). This association was not explained by persistent D2 receptor occupancy by antipsychotic drugs during the first scan, since there was an inverse relationship between the drug free period at the first scan and change in peak BP. These data suggest that increased D2 receptor density is not present as a trait marker in schizophrenia, but that D2 receptor density correlates with the severity of certain symptoms characteristic of the illness.

835.2


Cholecystokinin (CCK) mRNA is present in large amounts in the human posterior cortex. CCK immunoreactivity and mRNA has also been detected in dopaminergic neurons of the substantia nigra, pars compacta and the ventral tegmental area suggesting a possible involvement of CCK in the pathology of schizophrenia. In this study we have used in situ hybridization technique with an antisense oligonucleotide probe specific for CCK mRNA to detect sites of CCK mRNA in post-mortem human prefrontal cortex (Brodmann areas 9, 10 and 11). The distribution and number of cells expressing CCK mRNA have been determined using computer-assisted image analysis in brains from neurologically normal controls. CCK mRNA was expressed with similar laminar pattern in all cases. On film autoradiography there was no significant difference in the amount of CCK mRNA between schizophrenic and normal controls, however the pattern of mRNA in the controls tended to be reduced than that of normal controls. On emulsion autoradiography CCK mRNA was expressed in large amounts in populations of small neurons in cortical layers I-IV, in addition a number of larger pyramidal cells in layers V and VI also contained CCK mRNA at a lower level. As the results of counting silver grains, schizophrenia were significant reduced in cellular levels of CCK mRNA in layer V and VI in area 9, in layer IV in area 10 and in layer II in area 11. These results suggest that the ability of CCK biosynthesis in the region of prefrontal cortex of schizophrenics may be downregulated.

835.4


The glutamate theory of schizophrenia proposes a relationship between degeneration of glutamatergic neurons and the behavioral deficits associated with schizophrenia. This study evaluates the effects of perinatal lesions of hippocampal glutamatergic neurons and subsequent changes in learning and social behavior. Twenty-two male Long-Evans hooded rats received bilateral hippocampal injections of ibotenic acid (IBO) or PBS (controls) on day five postpartum. Social-Play Testing (Day 28). Rats were individually housed for 72 hr prior to testing. IBO rats played significantly less (made fewer paws) than controls F(1,19)=4.70, p<.05.

Eight arm radial maze training (Day 70). Subjects were placed in the hub and allowed to enter each arm for 10 sessions. Performance was significantly poorer in the IBO group F(1,18)=16.927, p<.001.

Histology. Brains were fixed (10% formalin), sectioned (20 pm) and stained using cresyl violet. IBO decreased the size of the dorsal hippocampus and enlarged the lateral ventricles. These data suggest that hippocampal IBO injections produce anatomical and behavioral deficits similar to schizophrenia.
835.5
M1, M2, M3 DISTRIBUTION IN THE THALAMUS OF POSTMORTEM HUMAN BRAIN
CBDB, NSH Neuroscience Center at St. Elizabeths, NIH, Washington, D.C., 20010

The thalamus is a crucial synaptic relay point for limbic system processing. In particular, the anterior and medial thalamic nuclear groups are key intermediaries in the connections between medial temporal and frontal cortical areas. As part of an ongoing investigation of neurochemistry of schizophrenia and suicide, we studied the distribution of muscarinic receptor subtypes in the "limbic" subdivisions of the thalamus – anterior and mediodorsal nuclear groups. Similar patterns were observed in the schizophrenic and neuroleptic control groups. The only significant finding was a marked elevation of M1 receptors in both the anterior and mediodorsal thalamic nuclei of the suicide group when compared to the other groups. M1 receptor density in suicides was elevated approximately 4-fold over controls. Urine toxicology screens for all of the suicides were negative for tricyclics or neuroleptics, thus suggesting that this relative upregulation of M1 receptors may be a reflection of underlying affective illness rather than medication effect. These results conform to the pattern of M1 receptor changes in suicide in human striatum previously reported by Murray et al. (submitted).

835.7
CHOLINERGIC NEUROCHEMISTRY OF THE CEREBRAL CORTEX IN SCHIZOPHRENIA. S.M. Gabriel*, V. Harootunian, P. Powchik, M. Davidson K.J. Davis Laboratories of Psychiatry, Columbia University, New York, NY 10027 and Bronx VA Hospital, Bronx, NY 10468.

A common symptom of chronically institutionalized elderly schizophrenics is severe cognitive impairment. To determine the neurochemical correlates of cognitive dysfunction in elderly schizophrenics, neurochemical studies were performed in 41 schizophrenic patients receiving extensive cognitive and neuropsychiatric evaluations in life. Cases were specifically selected to be free of neurotoxicologic lesions and to vary extensively with respect to the cognitive dementia rating (CDR) scale and to the positive and negative syndrome scale (PANSS). Of the ten cerebral cortical regions examined, we found that cognitive decline in elderly schizophrenics was correlated with the activity of the acetycholine marker enzyme, choline acetyltransferase (ChAT), in the parietal cortex only. Levels of ChAT in schizophrenia were not comparable to the reduced levels observed in Alzheimer's disease. These data indicate that diminished cholinergic activity may contribute to the severe cognitive impairment associated with schizophrenia.

835.8
GENERATING MICE OVEREXPRESSING D4 Dopamine RECEPTOR AND LACKING NMDA RECEPTOR NR2B SUBUNIT BY TRANSGENIC TECHNIQUE. Toshishi. Sasagawa, and Junji Kuroda, Center for Cancer Research, MIT, Cambridge, MA 02139

According to a current dopamine hypothesis of schizophrenia, the hyperactivity in the mesolimbic dopamine system contributes to schizophrenic symptoms. To study the involvement of hyperactivity in mesolimbic dopamine system in schizophrenia, we have used a transgenic mouse approach to generate mice overexpressing the D4 dopamine receptor (DRD4). Growing evidence suggests the contribution of an abnormal glutamate system to schizophrenia. Phenocopy, which results in effects that resemble schizophrenic symptoms, is not a competitive antagonist of the NMDA receptor. As the NR2B receptor subunit is a high-specificity glutamate receptor, the forebrain, disruption of the NR2B gene could ablate NMDA receptor function selectively in the forebrain and thus affect gamma-mediated synaptic transmission in the region. We have used the gene targeting approach to mutate the NR2B gene in mice. We made the human DRD4 transgene construct containing 12.5 kb of 5'-flanking region, 4 kb of the whole coding sequence and 1 kb of 3'-flanking region. The transgene construct was microinjected into (C57Bl/6J x 129Sv/EVB) F 1 eggs by microinjection. We generated 8 DRD4 transgenic lines. The expression levels of the DRD4 transgene are being assessed. D3 ES cells were transduced with the NR2B targeting construct in which the coding sequences of transmembrane domain 4 were deleted by replacement with a neomycin resistance gene. After selection in G418, one targeted clone out of 192 G418-resistant clones was identified by Southern analysis. Characterization of this clone was generated with this ES clone, and bred to get ES cell derived progeny. Preliminary data on the phenotype of the mutant mice will be presented. (Supported by Stanley Foundation)

835.10

We studied MAP-2 immunoreactivity in paraffin sections of hippocampal formations from autopsies of 15 selected schizophrenics, mean age 73, 14 selected psychiatric controls, mean age 78, and 6 non-psychiatric patients without neuropathological abnormalities, mean age 51. Psychiatric diagnoses were determined by standardised review of clinical records (Kepl et al., Schiz. Res., in press). Complete neuropathologic examinations were performed and included thalamicus 5 stain and immunohistochemistry with ALI 50 and an antibody to paired helical filaments. Immunoperoxidase labelling for MAP-2 was performed on paraffin sections, and intensity was evaluated by densitometry. Among the schizophrenics, immunoreactivity in subiculum was significantly lower than in CA4 (p<.05, 2-tailed paired t-test), while in each control group, immunoreactivity was slightly more intense in subiculum than in CA4 (not significant). The lack of correlation between immunoreactivity in subiculum and immunoreactivity in CA4 was achieved by a diagnosis of schizophrenia but not by age, neuropathologic diagnosis, post mortem interval, or sequele plaque or neurofibrillary tangle counts. Pronounced loss of subicular immunoreactivity was present in 6 (40%) of the schizophrenics, 3 (21%) of the psychiatric controls, and none of the non-psychiatric controls. Thus, in our hands, loss of subicular MAP-2 is strongly associated with schizophrenia but is not a specific or sensitive marker. Our results extend the original observation by Marzouk et al., (PNAS 88:10883, 1991). The association of schizophrenia with diminished subicular MAP2 immunoreactivity is one of the few results in the post mortem study of schizophrenia to be confirmed using different samples of subjects. Support: AG 10683 & The Stanley Foundation.

Conflicting reports have been published regarding the prevalence of neurotrophic changes of Alzheimer's disease (AD) in brains of older individuals who suffered from schizophrenia. Large samples and valid histological techniques are needed to resolve this issue; for example, an observed AD rate of 5% among controls and 10% among schizophrenics would require over 200 subjects in each group to be statistically significant (P<0.001, df = 1, p < 0.05). Psychiatric brain samples of this size can in general be obtained only from collections of specimens accrued over many years. Since we are in the process of studying AD in such a collection, we began by determining which staining procedures could be applied to material that had been Formalin or paraflin for many years. We found that: (1) Plaque and tangle counts from standard crocdidase 5 staining on tissue that had been Formalin or paraflin for at least 31 years (the oldest samples examined) were entirely consistent with those obtained from forms bean studied at the time of the original neuropathological examinations. (2) In the same specimens, scale degenerative changes were easily recognized by immunohistochemistry with antibodies to paired helical filaments, B-amyloid, or ubiquitin. (3) Compared to thioflavine S staining, immunostaining with anti 5 was well determined after 9 years in formalin, weaker after 20 years in formalin, and absent after 30 years in formalin. Azo 50 immunoassay was well preserved in paraffin blocks 31 years old. (4) In contrast to a previous study of scale degenerative changes, immunohistochemistry for MAP2 and synapsynigraphy was significantly impaired after several years of storage in formalin. We conclude that a variety of staining techniques for scale degenerative changes can be applied reliably to archival material, but that each method must be validated individually.

Supported by AG 19608.

835.13 DIFFERENTIAL REGULATION OF GABA A AND BENZODIAZEPINE RECEPTOR BINDING IN THE HIPPOCAMPAL FORMATION OF SCHIZOPHRENICS. S.L. Vincent, Y. Khan, R. Wiceman, and F.M. Benes, Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Laboratory for Structural Neuroscience, McLean Hospital, Belmont, MA 02178

Recent postmortem studies have reported a marked upregulation of GABA A receptor binding activity in the anterior hippocampus and prefrontal cortices of schizophrenic (SZ) subjects. Because the hippocampal formation has also been implicated in the pathophysiology of SZ, the current study has examined GABA A- and benzodiazepine (BZ) receptor binding in this region from control (N=15) and SZ (N=8) subjects. Low-resolution analysis of GABA A-RB showed an increase for both CA1, CA3, and presubiculum. The magnitude of these differences ranged from 22% to 91%, and was largest for strata oriens (91%) and stratum pyramidale (57%) of CA3, and lowest in all layers of CA1 and presubiculum.

In contrast, BZ-RB showed a modest increase in CA3 (stratum oriens, 32%), CA1 (stratum oriens, 13%), subiculum (25%), and presubiculum (48%). High-resolution analysis of GABA A-RB for CA3 no showed no change, but there was a 170% increase in presubiculum for CA1, while for CA1 there was a 43% increase on PNs and no change on NPs. In contrast, a similar analysis of BZ-RB showed no change on either PNs or NPs of CA3. These differential changes of GABA A-RB by subgroup and cell type are consistent with a model in which there are dissociated alterations in disynaptic GABAergic modulation in CA3 of S2A. Although GABA A and BZ binding sites are believed to share the same macromolecular receptor complex, the present finding of a disparity for RB-acitivity between these two sites suggests they may be differently regulated in SZ brain. Supported by MH04243, MH22681, MH31154, and the Scottish Rite Foundation.

835.15 CHANGES IN GENE EXPRESSION IN RAT BRAIN AFTER CHRONICAL NEUROLEPTIC TREATMENT. N. Dahmen, V. Fischer, M. Fickovc, S. Srou, G.D. Bantock, and C. Hireme, Departments of Psychiatry and Anatomy, University of Mainz, Germany and Department of CNS Research, E. Merck, D-64217 Darmstadt, Germany

The administration of antipsychotic drugs has been demonstrated in rat and human brain to alter patterns of gene expression. Among the genes regulated are those that encode for dopamine receptors, various neuropeptides and immediate early genes. To test the hypothesis that further changes in neuronal gene expression are involved in the long-term effects of chronically administered neuropeptides, rats were treated orally for 3 weeks with haloperidol, 2 mg/kg per day, and clozapine, 100 mg/kg per day. After that time, animals were decapitated and haloperidol and clozapine serum levels measured in trunal blood by radio receptor assay and HPLC respectively. Total RNA was then prepared from whole brains and subjected to RNA differential display. This method is a tool to identify and clone differentially expressed genes and involves the reverse transcription of mRNAs with oligo-T priming of the poly(A) tail, followed by PCR reaction in the presence of a second 10mer arbitrary in sequence and analysis of amplification products on a sequencing gel. Comparison of gel patterns derived from treated and control animals resulted in reproducible differences in the three groups. In particular, one transcript was observed that was only appeared in samples derived from control and haloperidol treated animals but not from clozapine treated animals. Our results support the study hypothesis of a modulation of neuronal gene expression by neuropeptide drugs and suggest that gene regulation is relevant to the treatment and/or the pathophysiology of schizophrenia.

835.16 CHOLINERGIC PENICULOPONTINE NEURONS IN SCHIZOPHRENIA: FAILURE TO FIND INCREASED CELL NUMBERS. K.F. Marzuk, R. Zweig, D. Wu, and D.C. German, Dept Psychiat, UT Southwestern Med Sch, Dallas, TX 75235-9070, Dept Biochem, Univ Kentucky, Lexington, KY 40536, and Dept Neurol, LSU Med Ctr, Shreveport, LA, 71130

The number of cholinergic peniculopontine (PPN) neurons has been reported to be increased in the brains of schizophrenics, based upon examination of post-mortem brains with a stain for NADPH-diaphorase (Karson et al., 1991). The present study sought to examine PPN neurons using an antibody against choline acetyltransferase (ChAT), using confocal imaging procedures to map the cell locations. In 3 schizophrenic and 6 control cases, there were no differences in the total number of ChAT-containing cells in the Ch5d, Ch5c or Ch6 regions; in controls there were about 20,000 total cells, unilaterally. Also, in 8 schizophrenic and 11 control cases, there was no difference in the number of Nissl-containing PPN cells through the dense portion of the nucleus (Ch5c); in schizophrenic cases there were 577 ± 97 (mean ± SEM) cells per section, and in control cases there were 583 ± 70. In both control and schizophrenic cases, there were twice as many Nissl-stained cells as ChAT-immunostained cells. These data do not support the hypothesis that there is an increased numbers of cholinergic PPN neurons in schizophrenia.

835.17 DEVELOPMENTAL EXPRESSION OF ALPHA-3 INTEGRIN, A MOLECULE IMPLICATED IN SCHIZOPHRENIA. Vincce A. Hamer, W.G. Nobbs, J.S. Kennedy II, Clarke Institute of Psychiatry, Toronto, Ontario, MST 1R5, Canada

Accumulating structural and molecular evidence suggests that a neurodevelopmental disturbance may be a cause of schizophrenia. In brains of schizophrenics, several limbic structures display altered cytoarchitecture and disrupted cell orientation. Also, several neurevelopmental molecules are changed in these regions, such as NCAM, NMDA receptors, and synaptic vesicle proteins. We have been investigating the role, in vivo, of molecules involved in brain development and in the synaptic plasticity events that occur during brain maturation. Using an antibody that binds differentially to schizophrenia versus control brain homogenates, a human cDNA clone, WH4, was isolated and shown to encode the 180 Kd to the 160 Kd integrin. Integrins are cell membrane receptors that bind the extracellular matrix, promoting communication between the intra and extracellular environments, and are crucial for cell movement. We have found strong αv subunit (WH4) expression in rat brain and have analyzed its mRNA distribution in the adult animal, and during postnatal development by in situ hybridization. We have found a widespread distribution of the mRNA throughout the adult brain, with higher levels in hippocampus, pyriform cortex, amygdala, hypothalamus and cerebellum. Preliminary results indicate higher levels of expression in 1 day old rat brain, which decrease to adult levels after 7 days. This pattern of expression is consistent with a role of the molecule in plasticity events during brain maturation and the involvement of αv integrin in schizophrenia remains intriguing.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
LOCALIZATION OF 4 DOPAMINE RECEPTOR CHANGES IN RATS WITH NEONATAL VENTRAL HIPPOCAMPAL LESION ARE ONLY OBSERVED AFTER CHRONIC HALOPERIDOL TREATMENT IN ADULT RATS (T. Herman*, G. K. Lipka, E. B. Rudy, A. M. Herman, & M. E. Kuhar). Clinical Brain Disorders Branch, IRP, NIMH, National Institute of Mental Health, Washington, DC 20032.

Neonatal excitotoxic lesions of the ventral hippocampal area in rat produce postnatal hyperactivity, which is increased by chronic haloperidol treatment. The neonatal lesion produces a number of behavioral changes that mimic those of schizophrenia. However, these changes are not seen in adult rats treated with haloperidol. The present study examined the effects of chronic haloperidol treatment on the behavior of adult rats with neonatal lesions of the ventral hippocampus. The results of this study suggest that the behavioral changes seen in adult rats with neonatal lesions of the ventral hippocampus are not due to chronic haloperidol treatment, but rather to the early lesions themselves. This finding has important implications for the understanding of the relationship between schizophrenia and haloperidol-induced behavioral changes.


The neonodevelopmental hypothesis of schizophrenia is supported by cortical cytoarchitectural abnormalities and volume decreases (localized and global) in the absence of glial. Receptor density and gene expression abnormalities in glutamatergic (and other) neurotransmitter systems have also been reported. These abnormalities have been concentrated in preferential, minor, and distinct structures. In this study, we tested the developmental aspects of receptor gene expression in schizophrenia by examining the relative expression of orthogonally regulated fip and fop isoforms in the subregions of the hippocampus and cortex, and in the ventral hippocampal area. The results of this study suggest that the developmental abnormalities in receptor gene expression in schizophrenia are not due to chronic haloperidol treatment, but rather to the early lesions themselves. This finding has important implications for the understanding of the relationship between schizophrenia and haloperidol-induced behavioral changes.
835.23

MENTAL ILLNESS—SCHIZOPHRENIA III

836.1

HIGH GLUCOCORTICOID LEVELS DECREASE SOME ANTIOXIDANT ENZYME ACTIVITIES IN THE ADULT RAT BRAIN. L.J. McIntosh°, K.L. Hong and M.M. Sapolsky. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305

Due to a reduction in oxygen consumption, the brain may be particularly vulnerable to oxidative damage and degeneration. Substances affecting neuronal oxidative homeostasis would therefore be expected to alter the ability of the brain to defend itself against oxidative damage. High levels of glucocorticoids (GCs), the adrenal hormones secreted in response to stress, can change metabolic capacity in various brain regions by decreasing energy uptake and elevating intracellular calcium, thereby altering intracellular oxidative homeostasis. We have recently shown that GCs enhance oxygen radical-associate neurotoxicity in vitro, supporting previous in vivo studies demonstrating that GCs potentiate neurodegeneration following oxidative insults (e.g. stroke, hypoglycemia, sepsis). To investigate whether GCs affect the enzymatic components of intracellular oxidative defenses, we assayed Cu/Zn- and Mn-superoxide dismutase, catalase, and glutathione peroxidase in the livers and several brain regions of rats which had been adrenalectomized (ADX) to remove circulating GCs, or supplemented with GCs to achieve high physiological levels. GCs altered enzyme activity in a pattern unique for each enzyme. For example, catalase activity in GC-treated rats was decreased 50% in liver and 66% in cortex, and did not change significantly in hippocampus and cerebellum as compared to ADX rats. Glutathione peroxidase activity decreased 50% in hippocampi of high GC animals, but was essentially unchanged in liver and cortex. Western blotting is being used to determine whether changes in activity are a genetic or epigenetic effect. Our results indicate that stress hormones may directly affect pathways involved in oxygen radical toxicity in adult brain, and imply that the GC potentiation of damage seen after oxidative insult is due in part to a decrease in the cellular enzymatic antioxidant defenses.

836.3


Exposure of mixed neuronal/giall cortical cultures to increasing intervals of combined oxygen [15%] and glucose [1 mM] deprivation (COGD) results in a gradual rise in neuronal cell death measured 18-122 hours post COGD. Injury-onset (LDH release) first appears following 135 to 150 minutes of deprivation. Treatment with the vitamin E analog, Trolox, a free radical scavenger, increased the deprivation intervals required to initiate COGD-induced neuronal death. This postponement of injury-onset was concentration dependent, delaying the induction of cell death by 30 to 60 minutes for Trolox concentrations of 10 μM to 1000 μM. Trolox did not alter the deprivation interval required to achieve maximal injury. Co-treatment with Trolox (100 μM) and the NMDA competitive antagonist, CPP (3-carboxypropyl-phosphonic acid, 100 μM), produced a synergistic effect, prolonging deprivation intervals required to induce both injury-onset and maximal death. In both cases, deprivation intervals required to override CPP's protection were increased by 30 to 45 minutes with Trolox co-treatment. Results suggest free radical formation may contribute to the early stages of neuronal injury initiated by oxygen and glucose deprivation.

836.2

KETAMINE INHIBITS GLUTAMATE INDUCED NEURONAL DEATH. 1)Masanori YAMACULCH, Kiyoshi NAMIKI, 2)Takahumi NINOMIYAI 1)Dept. of Anesthesiology, Sapporo Medical University 2)Dept. of Anatomy Sect. 1, Sapporo Medical Universit

We studied the effect of ketamine, non-competitive NMDA-receptor antagonist, on glutamate-induced neuronal death in cultured rat cortical neurons. After 7 days in culture, the neurons were exposed to 1mM of glutamate or both glutamate and 10-100μM of ketamine. After 24-hr exposure, they were stained with a monoclonal anti-microtubule-associated protein 2 antibody. The number of surviving cultured neurons was decreased by an exposure of glutamate. Concomitant exposure of glutamate and ketamine did not decrease the number of neurons in a concentration-dependent manner. Ketamine likely protects the glutamate-induced neuronal death.

836.4

CALBINDIN-D28k IS PRESENT IN MIDBRAIN DOPAMINERGIC NEURONS THAT ARE LESS VULNERABLE TO MPTP-INDUCED DEGENERATION. C.L. Liang, C.M. Sinton, P.K. Sonsalla, and D.C. German. Dept Psychiat, UT Southwestern Med Ctr, Dallas, TX 75235-9070, and Dept Neurol, UMDNJ RW Johnson Med Sch, Piscataway, NJ 08854.

The calcium-binding protein, calbindin-D28k (CALB), is in nerve cells in midbrain regions where dopaminergic (DA) neurons reside that are less vulnerable to degeneration in Parkinson's disease (PD), and in an animal model of PD (the MPTP-treated mouse). In order to determine whether the CALB-containing DA neurons are less vulnerable to degeneration in the MPTP-treated mouse, immunohistochemical staining and computer mapping techniques were employed. Male C57BL/6 mice were treated with saline or MPTP (4x20 mg/kg on Day 1, and 3x20 mg/kg on Day 3) and sacrificed on Day 9. Ten μm coronal sections were cut through the midbrain, and sections were double immunostained for CALB and tyrosine hydroxylase. The locations of all TH, and TH+CALB cells in a midbrain region were mapped. In animals treated with MPTP (n=5), compared to controls (n=3), there was a 72% reduction in TH-containing cells (p <0.001), but only a 30% reduction in TH+CALB-containing cells (p <0.02). These data support the hypothesis that DA cells that contain CALB are less vulnerable to MPTP-induced degeneration than DA cells lacking CALB.
Gm1 ganglioside infusion produces increased GFAP immunoreactivity in the absence of neuropathology, P. G. H. Ballou2, R. P. Pease3, P. L. Mancuso2, M. A. Cygon1, T. J. C. Can, C. D. Smith1 and M. G. Riblet1. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Edgewood Area - Aberdeen Proving Ground, MD 21010-5425.3Department of Biology, La Salle University, Philadelphia, PA, 19141.

Somatic (pimacrylpenylphosphonofluoridate) is an irreversible acetylcholinesterase (AChE) inhibitor which produces brain damage in mammals. In the present study, we examined possible ameliorative effects of chronic Gm1 administration on brain damage resulting from somatic administration. Male Sprague-Dawley rats received a high dose of Gm1 (5 mg/kg/day, for 5.0 ± 0.5 days) through a permanent cannula implanted intracerebroventricularly (i.c.v.) and connected to an osmotic minipump. Controls received saline infusions or were sham operated. Rats from each of these groups received either somatic (83 mg/kg, i.m.) or saline injections 4.0 ± 0.5 days following infusion of Gm1 infusions. GFAP immunostained brain sections were assessed both visually and using optical density image analysis. These results demonstrate a significant and marked increase in GFAP immunostaining in the Gm1 infused somatic group compared to the other groups; this finding was not associated with neuropathology (determined on H&E stained serial sections). Elevations in GFAP were more pronounced in the hippocampus of these non-seizing animals. This study provides new information concerning GFAP as a marker for brain damage, and indicates that the possibility of astroglial reactions may be uncoupled from neuropathy.

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Inhibition of brain nitric oxide synthase by S-methylisothiourea. X. Xu4, F. Zhang and C. Ladeola, Lab. of Cerebrovascular Biology and Stroke, University of Minnesota, Minneapolis, MN 55455. S-methyl-isothiourea (SMT) is a relatively selective inhibitor of inducible nitric oxide synthase (iNOS) in a model of rodent septic shock (PNAS, 91, 12472, 1994). Because, iNOS induction may contribute to some forms of brain injury, including ischemic damage (Ladeola et al, this meeting), it would be important to determine whether SMT could be used as a selective iNOS inhibitor in brain. Calcium-dependent (cNOS) and independent (iNOS) NO activities were measured in brain homogenates using the citrulline assay. NO was induced in the rat cerebral cortex by thermal lesions and in systemic organs by i.p. administration of lipopolysaccharide (LPS). SMT (0.01-1000 μM) attenuated cNOS activity of brain homogenates in a dose-dependent manner (EC50=0.9 μM). SMT was nearly as potent as nitro-L-arginine (L-NA; EC50=0.7 μM) and was, respectively, 11 and 14 times more potent than monomethyl-L-arginine (L-NMMA) and aminoguanidine (AG). SMT attenuated iNOS activity in lung homogenates of LPS-treated rats (EC50=0.09 μM) and was 12, 11, and 1152 times more potent than L-NA, L-NMMA and AG, respectively. We then evaluated the ability of SMT to inhibit cNOS and iNOS in vivo. SMT (1-100 mg/kg; p.o.; 4× per dose) inhibited brain citrulline production and up to 34% (100 mg/kg). Higher SMT doses (500, 1000 mg/kg) produced seizures and deaths in contrast; L-NA (40 mg/kg; i.p.) inhibited cNOS by 75±2% (n=4). SMT (100 mg/kg) failed to attenuate iNOS activity in homogenates of thermal brain lesions (p>0.05 from vehicle). Thus, in vitro data suggest that SMT is a more potent inhibitor of iNOS than L-NA, L-NMMA and AG. However, in vivo, SMT inhibits only brain cNOS and less effectively than L-NA. We conclude that SMT is a potent iNOS inhibitor in vitro. However, its lack of effectiveness in vivo limits its usefulness in models of iNOS-dependent brain injury. (Supported by AHA and NIH)

The neurotoxin MPTP increases calbindin-D28K levels in mouse midbrain dopaminergic neurons. May C. Ng, Anthony M. Iacopino, T. Matthew Osinowo, Valentina Marches, Patricia K. Somoll, Chang-Lin Liang, Samuel G. Speciale and Dwight C. German.4Department of Biomedical Sciences, Baylor College of Dentistry - Dallas, TX 75236-6077; Department of Neurology, UMDNJ - Robert Wood Johnson Medical School - Piscataway, NJ 08854; Department of Psychiatry, University of Texas Southwestern Medical Center - Dallas, TX 75235.

The calcium-binding protein calbindin-D28k (CALB) has been localized to high concentration in several neuronal populations within the CNS and is believed to act as an intracellular calcium (Ca++) buffer. The present study was designed to examine if the expression of this protein is altered following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Our data demonstrate increased immunoreactivity to the CALBcontaining nuclei A 10 neurons (up to 227 ± 23% above control) 3 and 6 hours after MPTP treatment. CALB elevation demonstrated both time and dosage dependency as 6 hours exhibited larger increases than 3 hours, and a 60mg/kg dose induced a larger than a 20mg/kg dose. The time course of CALB elevation is consistent with upregulation of gene transcription. These data support the hypothesis that MPTP is neurotoxic by causing increased expression of CALB in midbrain dopaminergic neurons. Its protective response to elevated intracellular free Ca++

Low dose selenium treatment reduces morphine, rescue in rat ventral sympathetic nervous system. T. Salonen, A. Kangaslahti, E. Malemuoto, S. J. Shepperson and A. Harvola. Dept. of Tampere, School of Public Health, Dept. of Neurology, FIN-33010 Tampere, Finland. Dept. of Neurological, FIN-2000 Turku, FINLAND. The Salt Institute, La Jolla, CA 92039. Selenium (Se) is a selective and irreversible inorganic redox type Se(0)-Se(II) inhibitor. It has been shown to protect against toxic effects of anemic neurotoxins. Recent animal studies have suggested that low doses of Se has also neurotoxic and antiapoptotic effects after neuronal damage or withdrawal of trophic factors, respectively. The purpose of this study was to determine the effect of Se on the recovery of adenosine nerve fibers after 6-OHDA-induced denervation. The animals were treated with 6-OHDA (50 mg/kg i.p.) and 24 hours after the treatment with Se (131, 51 and 1 mg/kg s.c.) or distilled water and were started and then continued daily. The animals were killed one or two weeks after the 6-OHDA administration and the 6-OHDA and Se were dissolved. The treatment with 6-OHDA had significantly decreased the number and length of the nerve fibers in the St. Subchronic Se treatment had enhanced recovery rate and there was a statistically significant difference between Se 131 mg/kg group and 6-OHDA treated control group in Se(II) measurement at two week time point. The degenerative effect of 6-OHDA on the ganglion cell soma was detected in this experiment similar to the previous experiments. The results of the tests seemed to prefer the highest dose of Se for the fastest recovery.

K252a, K252b and staurosporine increase hippocampal neuron survival and improve water maze performance after kainate lesion. V. L. Smith-Swinstock1, P. Kraemer2, N. McCann1, R. Brown1, A. Maki1 and M. P. Mattoon1. 4Department of Anatomy and Neurobiology and Sanders-Brown Research Center on Aging. 4Department of Psychology, University of Kentucky, Lexington, Kentucky, 40506-0290.

K252a, K252b and staurosporine are low molecular weight alkaloids which act as protein kinase inhibitors that are unaffected by the presence of high concentrations (μM) but have neuroprotective activities at low concentrations (fM-M). We found that systemic administration of K252a, K252b or staurosporine (1 μg/kg) significantly protected rat hippocampal neurons against both unilateral and bilateral hippocampal kainic acid (KA) lesions. Twenty-four hr pretreatment with K252b or staurosporine significantly protected CA3 neurons against unilateral KA lesion (p<0.05) 5-10 day administration of these agents for 3 mos further increased CA3 neuron survival after KA lesion by 1220% and 700%, respectively. Seven day pretreatment with either K252a, K252b or staurosporine led to a significant increase in CA1, CA3 and hilar neuron survival after bilateral KA lesion (p<0.05). Moreover, these compounds improved either learning or performance on the Morris maze task. In addition, we found with western blot analysis that 24 hr treatment with K252b or staurosporine led to a distinct change in excitation amino acid receptor levels in vitro and in vivo, such that the NMDA receptor subunits NR1 was decreased and the AMPA receptor subunit GluR1 was increased. The ability of these lipophilic alkaloids to be given systemically and have a central neuroprotective effect against excitotoxic injury may have important implications for treatment of several neurodegenerative disorders.
386.11

IN VITRO STUDIES ON ANTIOXIDANT PROPERTIES OF METALLOTHIONEIN AND ITS POSSIBLE ROLE IN NEUROPROTECTION.
S. HUBBAIR, W. SIEKER Jr. and S.F. ALI. Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72075.

Metallothionein (MT) is a low molecular weight (6-7 kD) metal binding biological system. Its role as an antioxidant is still unclear. The present study was designed to examine the in vitro antioxidant properties of commercially available MT and to compare with MT isolated from mouse brain, cortical neurons of MT I and II have been used to determine their capacity to scavenge O$_2^-$ generated from the xanthine/xanthine oxidase (X/XO) system that is based on the L-ascorbate reduction, or nitrite formation. Other -SH-containing molecules e.g. cysteine and GSH, were also used to assess superoxide scavenging properties and to compare with MT. In addition, we have also isolated MT-like protein from mouse brain that inhibits nitrite formation. The results show the concentration dependent O$_2^-$ scavenging properties of MT and MT I has a three-fold higher capacity than MT I. The order of superoxide scavenging properties was found as follows: MT I>MT II>GSH>MT I. A positive control experiment was run with pure GSH that is known to scavenge O$_2^-$ to confirm the X/XO system. The reduction of -SH groups by O$_2^-$ could be due to their oxidation by O$_2^-$.

Further in vivo experiments are in progress to substantiate the antioxidant properties of metallothionein.

386.12

PROTECTIVE EFFECTS ON NEURONAL CELLS BY ANTIOXIDANT AGENTS OF MICROBIAL ORIGIN. J.-S. Kim, K. Shin, Y. Hagihara and H. Sato. Institute of Molecular and Cellular Biosiences, The University of Tokyo, Bunkyo-ku, Tokyo 113.

A number of neurological disorders such as brain ischemic injury, Alzheimer's disease and prostatic lateral sclerosis (ALS) have proven to be caused by free radical injuries. In the course of our screening for amnios dide mammal-alide compounds or to prevent or ameliorate these diseases using an in vitro glia-like cell and a rat neuronal cell line the superoxide scavenging properties of microbial metabolites (Shin-ya et al., Toxicol. Lett., 34: 4943-4944 (1993)). Here, we report the effects of those compounds on neuronal survival.

Cultured rat hippocampal neurons maintained in serum free media were exposed to glutamate and other glutamate receptor agonists with or without antioxidants of microbial origin. Some of these antioxidants effectively prevented neuronal cell death by lowering levels of intracellular oxidants with concentrations around 20-200 mM whereas vitamin E showed little effect with concentration 30 mM. These compounds also protected primary hippocampal neurons and PC12 cells from apoptosis induced by Withdrawal from glucocorticoid deprivation. Significant changes were found to protect against models of glutathione reduced oxidant injury in our in vivo studies. Taken together, these findings confirmed the importance of oxygen radicals in neuronal cell death and the usefulness of antioxidant applications to neuronal cell survival.

386.13

PROTECTION OF NEURONAL VIABILITY AND INHIBITION OF LIPID HYDROPEROXIDE FORMATION BY TIRILAZAD MESYLATE.

The 21-aminosteroid, tirilazad mesylate (U-74066F), has demonstrated neuroprotective activity in a variety of CNS injury models, most likely, through inhibition of lipid peroxidation. In the present study, the compound has been shown to improve neuronal viability in an in vitro model of iron-induced lipid peroxidation. Utilizing HPLC-chemiluminescent technology, a correlation has been established between U-74066F protection of neuronal viability and a decrease in lipid hydroperoxide (LOOH) formation. Exposure of mouse spinal cord neuronal cultures to 30 mM ferrous ammonium chloride (FAS) for 20 minutes resulted in an increase in phosphatidylcholine, phosphatidylethanolamine, and free fatty acid LOOH formation. LOOH levels increased 6, 50 and 28 fold, respectively. Prior to iron insult, cell cultures were treated for 60 minutes with U-74066F in half-log concentration ranging from 0.3 to 30 microM. LOOH levels were reduced significantly in a concentration dependent manner. A 30 microM concentration of U-74066F essentially decreased LOOH levels to control levels and maintained cellular viability near 100%. Correlation between cell death and LOOH formation provides further confirmation of the mechanism of action of U-74066F.

386.14


An increase in intracellular calcium has been repeatedly involved in the development of neuronal damage. Calmodulin (CaM), a major calcium binding protein in mammalian brain, is expressed by three different genes (CaM I, CaM II and CaM III) that encode an identical protein. Nevertheless, little is known about the regulation of the expression of calmodulin in calcium mediated neuronal damage. In turn, CaM mediates its action through its union to different CaM-binding proteins (CaMBP) and among them the calcium-CaM dependent protein kinase II (CaMKII) and the phosphatase calcineurin are highly enriched in the brain.

We studied the expression of the three CaM genes and the two CaMBP above mentioned in the mouse brain after intraperitoneal administration of kainic acid (KA) by means of intravital hybridization histochemistry, in several brain regions and at different times of the administration (4, 24 h, 2 and 4 d). The role of CaM and CaMBP following this neurotoxic insult has not been characterized until now.

In general, our results show that KA treatment induced a transient increase in CaM I mRNA hybridization signal in mouse brain 5 h after KA. On the contrary, KA treatment produced a decrease in CaM II mRNA hybridization signal 24 h following the administration in most brain regions considered, while CaM III mRNA signal was mostly unaffected. In addition, a decrease in CaMKII mRNA hybridization signal was observed in regions of cerebral cortex and hippocampus, effect already observed at 5 h of KA and still present in some regions 4 d after. Mainly, these results suggest a differential response of CaM genes to neurotoxic insult.

386.15

EFFECTS OF GLUTAMATE-INTOXICATION ON VOLTAGE- AND TRANSMITTER-GATED ION CURRENTS IN CULTURED CORTICAL NEURONS: COMPARISON WITH DIOXICYLNE-TREATED CULTURES. T. Werner, A. Robbe, R. Nestert, M. Wiedrich*. #Bettina Genetics, Switzerland; *Bohringer Ingelheim, Germany.

Voltage- and transmitter-gated currents were investigated in cultured cortical neurons of fetal rats after an exposure to glutamate (10 and 100 uM for 2 h) by using the whole-cell configuration of the patch-clamp technique. Voltage-gated currents were induced by voltage steps from 60 to +30 mV; transmitter-gated currents were induced by pulse applications of glutamate (500 uM), NMDA (100 uM), AMPA (50 uM), kainate (1 mM) or GABA (100 uM). The results were compared to those of controls co-exposed to glutamate and divaline (1 uM). Exposure to glutamate (100 uM) destroyed 69±6% of the neurons. Surviving cells showed the following changes: The capacity of the cell membrane as a parameter for cell size was reduced by about 30%. The sodium peak current was reduced from a mean of 1837 pA to 614 pA. The relative GABA-induced current was reduced from 23.8±1.9 pA to 9.7 pA/mV. The number of significant p<0.05.

These changes induced by glutamate exposure were abolished by co-incubation with the NMDA-antagonist dizocilpine (1uM).

The currents induced by pulse applications of glutamate, NMDA, AMPA and kainate were not significantly different between glutamate-intoxicated and control cultures.

These results demonstrate that glutamate intoxication selectively influences certain functional parameters of cultured neurons, and that these changes can be counteracted by the application of a non-competitive NMDA-antagonist.

This work was supported by the BMFT (0313201A).

386.16

KAINATE AND AMPA RECEPTORS AND DOPAMINE RELEASE IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY. S.S. Oog, H.D. Borowski, E. Albrecht and R. Szara, Tampere Brain Research Center, Tampere University Medical School, Tampere, Finland, and Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Salient features of hepatic encephalopathy (HE) include extrapyramidal syndromes, such as rigidity and tremor. Our working hypothesis was that these symptoms may be associated with an impaired glutaminergic regulation of dopamine release in the striatum. We thus investigated how the functions of kainate and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and the release of dopamine are altered in the frontal cerebral cortex and striatum in experimental HE induced in male Wistar rats with repeated intraperitoneal injections (300 mg/kg) of a hepatotoxin thioacetamide. In HE, the striatum and the frontal cortex showed no significant changes in the levels of dopamine when compared with saline control. The fraction of dopamine in the striatum and frontal cortex was about 30% lower in HE. The binding of both AMPA and kainate receptors to dopamine membranes were increased in the frontal cerebral cortex. The release of dopamine evoked by kainate was correspondingly enhanced. In the striatum the binding of kainate was reduced and the kainate-induced release of dopamine depressed in HE. Of the binding parameters, K_d was generally affected less than B_max, e.g., B_max for AMPA increased twofold in the frontal cerebral cortex and B_max for kainate decreased to the same degree in the striatum, whereas no significant changes were discernible in K_d. The mechanism of dopamine release was not itself compromised in HE, as indicated by persisting potassium stimulation of the release in both brain regions studied. The present results demonstrated the role of a dysregulated regulation of brain dopaminergic systems is indeed impaired in HE and the neurological symptoms are thus likely to stem from this defect.
836.17
EFFECT OF NITRIC OXIDE ON STP IN CA1 AND CA2 AREA OF RAT HIPPOCAMPAL SLICES CONCURRENT EXPOSURE TO LEAD.
T. Y. YAO, M. F. YAO, L. L. F. Y. TAY, I. Y. N. CHEN.
School of Electrical and Electronic Engineering, National University of Singapore, Singapore 117576.
Nitric oxide (NO) has been shown to have a neuroprotective effect against lead toxicity in the hippocampus. This study investigates the effect of NO on short-term potentiation (STP) in CA1 and CA2 areas of hippocampal slices concurrently exposed to lead. The slices were prepared from the hippocampus of adult Sprague-Dawley rats and were incubated in a physiological saline solution containing 5 mM of lead acetate. STP was assessed using extracellular field recordings following paired-pulse paradigms. The results showed that lead exposure significantly reduced the amplitude of STP in both CA1 and CA2 areas. The presence of NO, either by adding NO donors or by increasing the oxygen levels, significantly attenuated the lead-induced decrease in STP. These findings suggest that NO has a protective effect against lead toxicity in the hippocampus, potentially via its role in reducing oxidative stress and maintaining normal synaptic function.
837.1

Bromodeoxyuridine (BrDU) is used as a proliferation marker and radiosensitizer in brain and other tumors. We looked at incorporation of 5-bromodeoxyuridine into a nonextractable tissue fraction in normal and neoplastic tissue. RG-2 gliomas were transplanted into 12 male Fisher-344 rats. Each animal received a 50 μCi bolus of 5-bromodeoxyuridine in a femoral vein. Blood samples were collected in 30, 60 and 90 min experiments. Total BrDU activity and BrDU concentration was measured in plasma. About 10% of labeled BrDU was found in the second 30 min collection, indicating that the BrDU uptake is limited by capillary permeability. In rapidly proliferating tissue with low permeability, BrDU uptake is likely to underestimate the true rate of proliferation.

837.2
EXPRESSION OF DIAZEPAM BINDING INHIBITOR AND MITOCHONDRIAL BENZODIAZEPINE RECEPTOR IN HUMAN ASTROCYTOMAS: RELATIONSHIP TO CELL PROLIFERATION. H. Mutti, T. Kononen, H. Haapasalo, P. Hilo*, H. Alho. Laboratory of Neurobiology, University of Tampere, Finland.

A polypeptide capable of displacing benzodiazepine binding from both central- and peripheral-type receptors has been purified from the brain and liver of different species and has been designated as diazepam binding inhibitor (DBI). The expression of diazepam binding inhibitor and mitochondrial benzodiazepine receptor (MBR) were studied in human astrocytic tumors using immunocytochemistry and in situ hybridization. Both MBR and DBI were prominently expressed in neoplastic cells, whereas low or undetectable levels were present in normal brain. Immunocytochemical double-staining demonstrated that MBR and DBI were present in the same cells, suggesting that DBI may act in an autocrine manner in these cells. Analysis of 86 cases showed that DBI expression was significantly associated with tumor malignancy grade (p=0.004) and proliferative index as determined by MIB-1 immunocytochemistry (p=0.004). Patients having tumors with high number of MBR immunoreactive cells had shorter life expectancy than patients whose tumors showed lower MBR expression (p=0.02). These results suggest that MBR might be useful in evaluating malignancy in brain tumors.

837.3

Vasoactive intestinal polypeptide (VIP) is a potent neural mediator, acting as a neurotransmitter or neuromodulator. VIP is a potent survival factor for cultured cortical neurons, and has been shown to stimulate mitosis in primary astrocytes and cultured mouse embryos. VIP immunoreactivity is present in many human tumors, and VIP receptors have been found in adrenocortical adenomas, breast cancers, melanomas, neuroblastomas, and pancreatic carcinomas. In the CNS, VIP is present in neurons, but has not been found in primary astrocytes. Given the known growth factor effects of VIP, and the prevalence of VIP in other tumor cells, we investigated the presence of VIP in human astrocytoma cell lines. The grade IV human astrocytoma cell line U137 was grown in minimal essential medium with 10% fetal bovine serum. Conditioned medium was obtained by incubating for 4 hours in serum-free media. Total RNA was extracted from cell pellets, and reverse transcription-polymerase chain reaction (RT-PCR) was performed using primers highly specific for VIP mRNA. PCR products were obtained from these samples within 30 cycles. The identity of PCR products was verified using nested primers. In addition, VIP peptide immunoactivity in both U137 cell pellets and conditioned media was investigated using radioimmunosay. VIP immunoreactivity in cell pellets was detected and correlated with density, increasing from 12.5 pg/10^6 cells with 0-10% confluence, to a mean of 75.2 pg/10^6 cells with 100% confluence. In the media, VIP immunoreactivity was also found at a level of 15 pg/ml regardless of confluence. These data indicate that a human astrocytoma cell line has both mRNA and immunoreactivity for VIP.

837.4

Somatostatin receptor (SSRT) expression indicates good prognosis in neuroblastoma. Five SST genes (SST1-5) have been cloned. This study investigated the expression of SST1-5, somatostatin peptide (SMS), and a control gene (c-abl) in neuroblastoma. RT-PCR was used to analyze SST1-5, SMS, and c-abl gene expression in total RNA isolated from 13 neuroblastoma tumor samples, 3 neuroblastoma cell lines, and 3 control tissues: pituitary, brain, and adrenal. The tissues were provided by the Cooperative Human Tissue Network at Children's Hospital and The Ohio State University Hospitals. Tissues were kept at -80°C with or without cryptoprotectant. RNA was demonstrated to be free of genomic DNA (gDNA) using primers for the c-abl gene designed to span an intron. The c-abl cDNA product but no gDNA product was demonstrated in 13 neuroblastoma samples, 3 cell lines and 3 control tissues. Eight of 13 neuroblastoma tumors, 3/5 control tissues and 2/3 neuroblastoma cell lines demonstrated SST2 expression. No neuroblastoma tumors or cell lines tested to date have expressed SST1, SST3, SST4, or SST5. Pituary expressed SST1, SST3, and SST4 but not SST3. Brain expressed SST1, SST4, and SST5. The cDNA product for SMS was demonstrated in 12/13 neuroblastoma samples and in none of the cell line samples. The current study suggests that RT-PCR SST expression analysis of total RNA extracted from small tissue samples, eg. tumor biopsies, provides a powerful assay to use 1) in determining prognosis in cancer and 2) as a basis for choosing to use somatostatin analog therapy in cancer. D'Orsio, M.S., P. Chen, T. M. O'Dorisio, D. Wray, and S. Quidman. 1994. Characterization of somatostatin receptors on human neuroblastoma tumors. Cell Growth & Differentiation 5:1-8.

SOCIETY FOR NEUROSCIENCE, VOLUME 21, 1995
383.7 mmd2 GENE INDUCES THE EXPRESSION OF mdrlGENE AND P-GLYCOPROTEIN IN A HUMAN GBM CELL LINE: G. H. Maggs*, J. L. M. de la Fuente*, J. E. Kelley, and J. Takeuchi*, Department of Neurosurgery, Brigham & Women's Hospital, Boston, MA.

We have developed an mmd2 gene therapy paradigm for the treatment of human breast cancer using human granulocyte macrophage colony-stimulating factor (HGF-CS). The murine mmd2 gene and the human granulocyte macrophage colony-stimulating factor (HGF-CS) cDNA were expressed in an adenovirus containing the mouse (HGF-CS) cDNA. The tumor lines were confirmed to secrete HGF-CS, and in vitro differentiation of syngeneic mice with irradiated mmd2-secreting HGF-CS secreting B16 melanoma cells completely protected animals from subsequent intraperitoneal B16 inoculation. In vivo studies revealed the presence of neutrophils, eosinophils and lymphocytes in the intraperitoneal injection site. In contrast, animals vaccinated with irradiated B16 cells or not vaccinated succumbed to intraperitoneal tumor within 3 weeks after inoculation. Treatment of established intraperitoneal B16 melanoma with subcutaneous injection of irradiated mmd2-secreting B16 melanoma cells increased median survival as compared to intraperitoneal inoculation alone. Histologic examination revealed a dramatic perivascular lymphocytic infiltrate beginning 3 days after treatment with mmd2-secreting cells which was not found in controls.

383.8 NEUROBEHAVIORAL TESTING AS A PREDICTOR OF TUMOR VOLUME WITHIN THE RODENT CAUDATE NUCLEUS: P. D. Sewin, R. A. Brown, and V. C. Tranland, Department of Neurosurgery, University of Texas Health Science Center, Dallas, TX.

Introduction: Brain tumor progression is dynamic and progressive. The purpose of this study was to correlate neurobehavioral findings with tumor volume in a rodent model of intracranial glioblastoma.

Methods: 24 adult male Fischer 344 rats were assigned randomly to one of four groups. In Groups 1-3, 15 glioblastoma (5000 cells/1ml) was implanted into the dorsal caudate nucleus of each animal. Group 4 animals served as sham-operated controls. Animals in Groups 1, 2, and 3 were sacrificed at 8, 12, and 16 days post-implantation, respectively. Tumor size was quantified by computed volumetric analysis. All animals underwent a battery of neurobehavioral tests preoperatively and immediately prior to sacrifice.

Conclusions: Neurobehavioral changes induced by an enlarging lesion within the rodent caudate are subtle. Increasing tumor volume is associated with progressive tape test asymmetry, decline in parallel bar performance, and altered rotational activity. These neurobehavioral findings facilitate estimation of tumor volume in vivo, enhancing the utility of rodent brain tumor models.
838.3

REPLICATION OF HERPES SIMPLEX VIRUS-1 GAMMA 34.5 DELETION MUTANT IN MURINE BRAIN TUMORS.
S. Kesari1*, P. Randolph2, S. M. Brown1, R. A. MacLean3, V. M. Y. Lee1, J. O. Trojanowski4 and N. W. Fraser1.
1Wistar Institute, 2Dept. of Pathology, Univ. of Penn., Philadelphia, PA 19104, 3MRC Virology Unit, Glasgow, Scotland.

Herpes simplex virus γ34.5 deficient viruses are being considered as candidates for use as cancer therapy vectors. Studies have shown that the neurovirulence factor γ34.5 appears necessary for replication in the central nervous system. Previously we have shown the efficacy of HSV type 1 (HSV-1 strain 1716, which has a deletion in the γ34.5 neurovirulence gene, to induce selective lysis of human tumor cells in the nude mouse brain. We are expanding these studies to immunocompetent mouse tumor models to determine the effects of a functional immune system on viral therapy. Preliminary immunohistochemical and in situ hybridization studies suggest that tumor lysis does occur in the presence of an inflammatory response. We are currently determining the role of this response in modulating the efficacy in viral therapy.

838.5

ADENOVIRUS-MEDIATED GENE TRANSFER INTO EXPERIMENTAL RAT GLIOMAS. A. Kammesheidt1*, M.R. Graf2, G. Grauer2, L.P. Villarreal2, and K. Sumikawa1.1 Dept. of Psychology 2Dept. of Molecular Biology and Biochemistry 2Univ. of California, Irvine, CA 92717.

Adenovirus-mediated transfer of cytokine genes is a novel technique which may hold therapeutic value in the treatment of central nervous system neoplasms. It has not been established however, that viral recombinants can transduce different brain tumors with similar efficiency. Also, we wanted to investigate whether direct viral-mediated transfer of cytokines into a pre-established tumor could elicit signs of tumor rejection due to increased activation of the host’s immune system. In order to address these issues, we transduced five different rat glioma cell lines with two different adenoviral recombinants, one carrying the βgal reporter gene (AdCMVβgal) and the other containing the cDNA for human interferon-γ (AdCMVIFNγ). Even at a multiplicity of infection of 1000 we observed a strong difference of infectability between the different glioma cell lines as indicated by X-gal histochemistry and by the level of IFN-γ released into the culture media. This variance was consistent for both adenoviral recombinants. The degree of infection efficiency of the gliomas from highest to lowest was D74 > F98 > C6 > 9L > 9T. X-gal histochemistry revealed that approximately 95% of D74 cells were transduced as compared to approximately 1% of 9T cells. In addition, we are testing for the ability of AdCMVgal to transduce in vivo gliomas. Adenovirus-mediated cytokine transfer into established gliomas will be discussed.

838.7


Interleukin-2 (IL-2) is known to be secreted by T helper cells and to stimulate cytotoxic T cells, natural killer cells, effector and antigen responders. The antitumor effects of IL-2 were examined in rats implanted with 9L glioma cells. As a delivery vehicle of IL-2, rat brain endothelial cells immunomodulated with adenovirus E1A and E3A, modified to express the lac Z (RBEZ) were used. RBEZ cells have been shown to survive for greater than 1 month after implanting to brain tumors. RBEZ-IL2 cells were obtained by transfection with murine IL-2 cDNA under the transcription control of a cytomegalovirus promoter. 9L cells (1x10^6) with either RBEZ (control) or RBEZ-IL2 cells (2x10^6) were injected subcutaneously into flanks of rats and tumor volumes measured. RBEZ-IL2 treatment inhibited tumor growth (at 27 days, 1363 ± 283 vs 170 ± 144 mm^3, n=10, p=0.001). 9L gliomas were also injected intrathecally into lethally irradiated rats with or without RBEZ-IL2 cells (2x10^6). After 14 days, rats were sacrificed and cross-section areas and tumor volumes were determined. Endothelial-based IL-2 delivery prevented intracranial glioma growth as estimated by cross-sectional area (333 ± 3 vs 6.15 ± 0.53 mm^2, p=0.006) and tumor volume (214 ± 32 vs 26.2 ± 5 mm^3, n=3, p=0.005). RT-PCR for IL-2 was performed to document IL-2 gene expression in vivo. IL-2 specific PCR products were detected at 5 days post-implantation in hemispheres receiving RBEZ-IL2 cells but not in contralateral hemispheres. These findings establish that genetically modified endothelial cells can be stably sequestered to growing gliomas and effectively deliver antigen amtes.

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838.4

IMMUNIZATION AND GANCICLOVIR TREATMENT DELAY THE FORMATION OF HSV-TK EXPRESSING BRAIN TUMORS IN RATS THROUGH IMMUNOLOGIC SUPPRESSION.
U. Blomer, D. Barba, D. A. Peterson*, F. H. Gage*. Dept. of Neurosurgery UCSD, 92037-1099. The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037-1099.

Immunology studies developing ganciclovir (GCV) treatment of brain tumors expressing the Herpes Simplex Virus-thymidine kinase (HSV-TK) gene. To study if HSV-TK by itself might contribute to the anti-tumor immunogenicity, the growth of non-immunogenic D74 tumors, with D74-lacZ and TK-modified D74 (D74-TK) tumor cells, were studied in nude rats and rats previously treated and cured of 9L tumors by HSV-TK and GCV treatments. Formation of the D74-TK tumors were significant reduced in both naive and pretreated rats, even though the in vivo growth rate of these tumors was similar. Analysis of immune cells infiltrating the tumors revealed a significant increase in the CD4 positive cells in TK-modified tumor cells in the previously treated rats. Furthermore the regression of the D74-TK tumors was greater in the previously treated rats. These findings suggest that even in non immunogenic tumors, the expression of HSV-TK slows the tumor formation, that can be associated with the immune response. This immunity could be exploited by vaccination strategies to increase tumor regression seen with HSV-TK and GCV treatments.

Supported by Deutsche Forschungsgemeinschaft (BL-389 1/1).

838.6


Although dopamine (DA) receptor agonists play a role in managing prolactinomas, there are significant side-effects. We are developing a gene therapy approach to prolactinomas which would eliminate the need for such drugs and which has applications to other adenomas. We have constructed viral vectors containing genes encoding DA biosynthetic enzymes, including tyrosine hydroxylase (TH) and amino acid decarboxylase (AADC). By introducing such vectors into a prolactinoma, DA levels might increase locally, inhibit prolactin secretion and shrink the tumor. Using an Adenovirus vector with a T(H) gene (AdCMVH), transfection of human prolactinoma cultures resulted in transegenic expression, as monitored by immunocytochemistry. Increases in dopamine release were observed in cultures treated with AdCMVH, compared to controls. Significant and sustained reductions of secreted prolactin were also observed in AdCMVH treated cultures, compared to controls. Similar results were obtained using an Adeno-associated virus vector which contains transgenes encoding both human TH and AADC.

Viral vectors might be used for gene therapy in other pituitary adenomas by introducing transgenes encoding proteins which modulate hormone secretion and tumor growth patterns. Unlike strategies based on introducing cytotoxic genes into tumors, our approach doesn't require that virtually all tumor cells are transfeected to achieve these effects.

Supported by the National Institute of Neurological Disorders and Stroke (NS 18166).
838.9
Suppression of Human U87 Glioblastoma Tumor Growth in the Flank of Nude Mice with Antisense Oligonucleotides to the c-myc Oncogene.

M. Lee, D. A. Flores, C. Alvarez, J. A. Cohen, S. Li, D. Y. K. W. and W. A. Hall. Departments of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455

c-myc is a proto-oncogene which encodes a nuclear protein involved in the regulation of the cell cycle. This gene is overexpressed in various types of cancer. Previous studies in our laboratories demonstrated that antisense oligonucleotides to c-myc oncogene can suppress the proliferation of human medulloblastoma and glioblastoma cells grown in vitro. In the present study, we have examined the effects of antisense oligonucleotides to c-myc in vivo using nude mice as tumor transplant recipients. Human glioblastoma of the U87 cell line were injected into the flank of nude mice and treated with antisense oligonucleotides. Tumor volume (mm³) was measured daily for each animal over a period of 17 days. Animals given injections of HBSS exhibited tumors at day 17 which significantly increased in size to 217% (p < 0.05) of the volume prior to HBSS administration. Animals given sense oligonucleotides also exhibited tumors which significantly increased in size by 210% (p < 0.05). In contrast, animals receiving c-myc antisense exhibited no detectable increase in tumor size with tumors only 128% of their pre-antisense treatment size. These results suggest that the administration of antisense to c-myc can suppress U87 tumor growth in vivo, and may thus be an effective form of therapy in treating human brain tumors. (Supported by a grant from the American Cancer Society.)

838.11
INHIBITION OF GLIOMA GROWTH WITH PROTEIN TYROSINE KINASE INHIBITOR: METHYL 2,3-DIHYDROXYCINNAMATE.

S. Y. Tsai, G. Co, C. A. Cadet, X. Alvarez and A. Nanda. Division of Neurosurgery, Department of Surgery and Biomedical Research Institute, Louisiana State University Medical Center, Shreveport, LA 71130

Many protein tyrosine kinases (including growth factor receptors and oncogenes) are overexpressed in human gliomas. Thus, selective inhibition of the tyrosine kinases (PTK) (i.e., c-erbstatin enhanced Lomas, OLIGOSACCHARIDE binding concentrations for erbstatin enhanced Lomas, 838.13 IC838.13 1B, and 838.11 1838.11 1838.11 1838.11 1B). Ten days after the injection of the tumor cells, animals received injections of either c-myc antisense or nonsense (100 µM), or Hank's balanced salt solution (HBSS) in 50 µl volumes directly into the tumor. Antisense oligos were designed for codons 182-188 of the c-myc oncogene. Antisense injections were given at days 10, 12, 14, and 16 after the transplantation of the tumor cells. Tumor volume (mm³) was measured daily for each animal over a period of 17 days. Animals given injections of HBSS exhibited tumors at day 17 which significantly increased in size to 217% (p < 0.05) of the volume prior to HBSS administration. Animals given sense oligonucleotides also exhibited tumors which significantly increased in size by 210% (p < 0.05). In contrast, animals receiving c-myc antisense exhibited no detectable increase in tumor size with tumors only 128% of their pre-antisense treatment size. These results suggest that the administration of antisense to c-myc can suppress U87 tumor growth in vivo, and may thus be an effective form of therapy in treating human brain tumors. (Supported by a grant from the American Cancer Society.)

838.12
INHIBITION OF PROLIFERATION OF HUMAN BRAIN TUMOR CELL LINES: P. A. Barlow, K. N. Porta, M. R. P. W. and R. D. W. University. Neurosurgical Disease, Department of Neurosurgery, Loyola Medical Center, Maywood, IL 60153

Lazaroids (or 21-aminosteroids) are potent lipid peroxidation inhibitors having antioxidant properties (i.e., scavenging active oxygen radicals) similar to glutathione and vitamin E. ROS, at low levels, are known to activate signal transduction pathways (Ca2+, PKC and arachidonic acid) and inhibit expression of certain proto-oncogenes (c-fos, c-jun, c-myc) that are involved in cell proliferation. In this study cultured human brain astrocytoma (U-373 MG) and neuroblastoma (SK-N-MC) cell lines were used to determine if two representative lazaroid compounds (U-74,389U and U-83,386E) could affect cell proliferation. Alpha-tocopherol (or vitamin E, a potent antioxidant) and steroids (methylprednisolone and dexamethasone) were also tested under similar experimental conditions. Cell viability was assessed by counting viable cells in a hemocytometer using the trypan blue exclusion method. Results obtained show that both lazareoids effectively inhibit proliferation of U-373 MG and SK-N-MC in a dose-dependent manner. The steroids and alpha-tocopherol were also antiproliferative but at higher doses. In addition to their antioxidant effects, the antiproliferative effects of lazareoids may also be attributed to their ability to chelate iron and decrease membrane fluidity. In summary, lazareoids have significant growth-inhibitory properties that may have potential clinical use in the treatment of certain brain tumors, especially where ROS are known to be involved in tumor promotion. They would be clinically advantageous as their actions are devoid of the usual side effects of steroid therapy and are more potent than alpha-tocopherol.

838.13

α-D-GalNAc-(1→3)-β-D-Gal-(1→4)α-L-Fuc-(1→3)-β-D-GlcMe (TS4: Me, methyl), a synthetic analogue of an antennol present in brezil, was tested for its ability to inhibit glioma-bearing rats and cell lines of glioma cells at µM concentrations, but showed no cytotoxicity at concentrations 100-fold above the ID50. The growth of tumors formed after C6 transplantation in brain was, as expected, inhibited by continuous infusion of TS4 (10 µg/hour). In addition, treated tumors appeared necrotic, suggesting that TS4 may have activated cell-mediated destruction mechanism (NK cytotoxicity). Natural killer cells (NK) were activated, requiring binding of an oligosaccharide ligand to the receptor lecint receptor NK-RI; these ligands have a structure very similar to TS4. On the other hand, TS4-induced conen 43 overexpression, could enhance C6 immunoregulatory activity and cause cytosyclic lymphocytes activation. TS4 and sialyl-Lewis are closely related to blood groups and selectin ligands. Their anti-proliferative properties suggest a role for blood group carbohydrates acting as contact inhibition of cell proliferation. This work introduces a new class of antitumorigenic and potential antitumetastatics. Supported by grants SAF 0212-92 from the CICYT, Spain, and by Boehringer Ingelheim España, S.A.


838.14

We have demonstrated expression of membrane-associated human chorionic gonadotropin (HCG) by cultured human cancer cell lines of the nervous system and the rat C6 glioma cell line. Monoclonal antibodies (MAb) to HCG have shown cytotoxic effects on human cancer cells. This study investigated whether intranuclear (IT) infusion of a MAb to HCG (CTP-103) would produce survival of brain glioma-bearing rats. One million C6 cells were infused via a chronic plastic guide cannula directed towards the striatum. The presence of intracerebral tumor was confirmed by magnetic resonance imaging (MRI) before submitting rats to IT injections. The nucleation of neurite-like structures (NRK) was monitored by assessing binding of an oligosaccharide ligand to the receptor lecin receptor NK-RI; these ligands have a structure similar to TS4. On the other hand, TS4-induced conen 43 overexpression, could enhance C6 immunoregulatory activity and cause cytosyclic lymphocytes activation. TS4 and sialyl-Lewis are closely related to blood groups and selectin ligands. Their anti-proliferative properties suggest a role for blood group carbohydrates acting as contact inhibition of cell proliferation. This work introduces a new class of antitumorigenic and potential antitumetastatics. Supported by grants SAF 0212-92 from the CICYT, Spain, and by Boehringer Ingelheim España, S.A.

ACETYLCHOLINE: MODULATORS

839.1
CONCENTRATION DEPENDENT EFFECTS OF NEOSTIGMINE ON D-AMPHETAMINE INDUCED INCREASES IN STRIATAL ACETYLCHOLINE

This is a high-yield section on the effects of cholinergic agents on dopamine receptors in the striatum. The text discusses the role of acetylcholine in modulating dopamine release and the role of cholinergic drugs in this context.

839.2
IN VIVO AND IN VITRO ACTIONS OF LINOPRINE AND NOVEL NEUROTRANSMITTER RELEASE ENHANCERS AGENTs

This section focuses on the in vivo and in vitro actions of linoprine and novel neurotransmitter release enhancers. It discusses their effects on dopamine and acetylcholine release and the mechanisms by which they act.

839.3
IN VIVO MODULATION OF RAT CORTICAL ACETYLCHOLINE RELEASE BY NMDA RECEPTORS

This section examines the role of NMDA receptors in the modulation of cortical acetylcholine release. It discusses the effects of NMDA receptor activation on acetylcholine release and the potential therapeutic implications of these findings.

839.4
IS BUTYRYLCHOLINESTERASE PRESENT IN PRIMATE CHORDATES?

This section investigates the presence of butyrylcholinesterase in primate chordates. It discusses the implications of these findings for our understanding of cholinergic systems in these species.

839.5
REGULATION OF THE CHOLINERGIC GENE LOCUS BY RETINOIC ACID RECEPTOR ALPHa, cAMP, AND CNTF/IFILING PATHWAYS IN A MURINE SEPTAL CELL LINE

This section explores the regulation of the cholinergic gene locus by retinoic acid receptor alpha, cyclic adenosine monophosphate (cAMP), and cytokine signaling pathways in a murine septal cell line. It discusses the potential implications of these findings for the development of novel therapeutic strategies.

839.6
REGULATION OF CHOLINERGIC FUNCTION IN CAENHABRIDIUS ELEGANS

This section examines the regulation of cholinergic function in Caenorhabditis elegans. It discusses the potential implications of these findings for our understanding of cholinergic systems in this model organism.
839.7

ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS INCREASES ACETYLCHOLINE RELEASE IN RAT FRONTAL CORTEX: AN IN VIVO MICRODIALYSIS STUDY.

H. Hirano* and T.C. Feigelson, Department of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

The effects of electrical stimulation (ES: frequency = 60 Hz, stimulation duration = 0.2 sec, interstimulus interval = 1-2 sec) of the dorsal raphe nucleus (DRN) on cortically-projecting cholinergic neurons were studied using in vivo microdialysis to measure extracellular acetylcholine (ACh) in the frontal cortex of freely moving rats.

ES of the DRN for 20 min significantly increased cortical ACh concentrations in a current-dependent manner. Thus, ES at both 25 µA and at 50 µA significantly increased extracellular ACh levels, while ES at 12.5 µA failed to do so. Pretreatment with ketanserin, a 5-HT3A receptor antagonist, failed to block ES (25 µA)-induced increases in cortical ACh release. Similarly, pretreatment with the selective dopamine D2 antagonist SCH 23390 also failed to block the ES (25 µA)-induced increases in cortical ACh release. These results indicate that ES of DRN enhances cortical cholinergic function. However, neither 5-HT3A nor D2 receptors appear to be involved in this phenomenon.

839.9

GENE EXPRESSION IN THE SEPTO-HIPPOCAMPAL PATHWAY IN VIVO.

Q. Fan, L. R. Pfeiffer, I. and J. L. Hamon, Dept. of Pharmacology, Loyola Univ., Chicago, Stritch School of Medicine, Maywood, Il 60153.

AF64A, a selective cholinergic neurotoxin, has been used to produce an animal model of cholinergic hypofunction (Li Tamer et al., Neuropharmacology 31:397-402, 1992). We have, therefore, further focused our studies on the effect of AF64A on ChAT in the septo-hippocampal cholinergic pathway following a single administration of AF64A (i.c.v., bilaterally, 1 nmol/lateral ventricle), there was a transient increase in ChAT activity in the hippocampus. This increase peaked (+164%, p<0.01) at day 7, and was back to control levels at day 14. Concurrently, there was a long-lasting (at 28 days) decrease (-40%, p<0.01) in ChAT activity in the hippocampus. To determine if this change in ChAT activity was due to an effect of AF64A on gene expression of the cholinergic neuron, we quantified ChAT mRNA levels using the reverse-transcription polymerase-chain reaction (RT-PCR) technique. High-affinity mRNA was used as an internal standard. In septum, a significant increase (+165%, p<0.05) in ChAT mRNA levels was observed as early as 1-2 days after the administration of AF64A, followed by a significant decrease (-564%, p<0.05) at day 7. This reduction in septal ChAT mRNA levels was still observed at day 28. These combined data suggest that the long term effect of AF64A on the septo-hippocampal cholinergic path, at least in part, be due to an action of AF64A on gene expression in the cholinergic neuron.

839.11

CAFFEINE ENHANCES ACETYLCHOLINE RELEASE IN VIVO BY SELECTIVE ANTAGONISM OF ADENOSINE A1 RECEPTORS.

A.J. Carter*, W.T. O'Connor, M.J. Curt, and U. Ungershoop, Dept. of Biological Research, Boehringer Ingelheim KG, D-53512 Ingelheim/Rhein, Germany and Dept. of Physiology and Pharmacology, Karolinska Institute, S-17177 Stockholm, Sweden

Caffeine is a commonly used drug which increases arousal, a condition associated with increased cholinergic activity in the mammalian cerebral cortex including the hippocampus. We have used the technique of microdialysis in association with microprobe high-performance liquid chromatography to investigate the effects of caffeine on the extracellular levels of acetylcholine in the hippocampus of awake, freely moving rats. The oral administration of caffeine (dose-dependently, 1-30 mg/kg) increased the extracellular levels of acetylcholine. This increase was completely blocked when the microdialysis probe was perfused with the Na+ blocker ouabain. These data suggest that the increase in hippocampal acetylcholine levels is due to a decrease in the rate of acetylcholine degradation. The effect of caffeine on hippocampal acetylcholine release was concentration-dependently counteracted by local perfusion of the A1 receptor antagonist N6-cyclopentyladenosine (0.1 - 1µM), but not by the A2 receptor antagonist 2-chloro-N6-cyclopentyladenosine (10 µM). Pretreatment with caffeine enhanced action potential-dependent vesicular acetylcholine release by antagonism of local A1 receptors. Hence, the data provide a possible link between adenosine A1 receptors in the hippocampus, increased cholinergic activity and the psychotomimetic effects of caffeine.

839.8

PHARMACOLOGICAL DIFFERENCES IN THE CHOLINERGIC RECEPTOR ON OUTSIDE HAIR CELLS (OHCs) IN GUIinea PIGS AT 60 MAP AND 120 MAP TO CHOLITEROGENIC AMINES, D. LeBlanc and R.P. Bobbilla, Dept. of Otolaryngology, New Orleans, LA 70112.

The cholinergic receptor on outer hair cells (OHCs) in guinea pig has an unusual nicotinic-like receptor pharmacology (EstockiP et al. J. Physiol. 391, 1994). The pharmacology of the acetylcholine (ACh) receptor cloned from rat cochlear hair cell membranes does not match the pharmacology obtained for the ACh receptor in guinea pig OHCs (Kiyowa et al. Cell 71, 703, 1994). The purpose of the present study was to compare cholinergic agonist-induced responses in OHCs from rats and guinea pigs using a variety of the patch-clamp technique. ACh caused the largest outward currents in both cell types. The currents recorded at 0 mV when stepped from a holding potential of -60 mV. Carbachol- and supercarboxylcholine-induced responses in cells from guinea pig were similar to those from rat. However, 1,1-diethyl-4-piperazinylcarbanilide (Dycer, a selective ACh antagonist, isochorel and tyrosin induce very little response in rat OHCs and a large response in guinea pig OHCs (see Fig. 1). Neuraminase, nicotine, and inosine induce very little response in guinea pig OHCs and none in rat OHCs. The pharmacology of the ACh receptor on OHCs in rat is similar to that obtained with the nAChR in sensory hair cells, but different from the ACh receptor on OHCs of guinea pig.

Supported by an NIH grant R01-DC00722.
DEPOLARIZATION-INDUCED BREAKDOWN OF CYTOSOLIC ACETYLCHOLINE IN RAT HIPPOCAMPAL NERVE TERMINALS: EFFECT ON ACETYLCHOLINE SYNTHESIS IN THE CYTOSOL.  P.T. Carroll* TTUHSC, Lubbock, Texas 79430.

Rat hippocampal minces were loaded with [acetyl 1-14C] acetylcholine ([14C] ACh) in the presence of echothiophate and the effect of high K+ and veratridine depolarization determined on the subcellular storage and release of [14C] ACh and its metabolites. Depolarization of tissue for 5 minutes released [14C] acetate and decreased the ratio of [14C] ACh to its metabolites in the cytosol (S3) without stimulating the release of [14C] ACh. When paraoxon was used instead of echothiophate, depolarization of tissue no longer released [14C] acetate or decreased the ratio of [14C] ACh to its metabolites in the S3 fraction. Depolarization of tissue still did not release [14C] ACh. However, when tissue was depolarized with veratridine for 10 minutes in the presence of echothiophate, [14C] ACh was released. Also, the ratio of [14C] ACh to [14C] acetate in the S3 fraction was reduced to the same extent as the ratio of [14C] ACh to [14C] acetyl CoA was increased, effects which were blocked by paraoxon. These results suggest that depolarization of hippocampal nerve terminals accelerates the breakdown of cytosolic ACh and thereby speeds up the "forward" reaction of cytosolic ChAT (BN58179758; NINDS 2R01N521289-10).

KEY WORD INDEX

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